THE CARDIOVASCULAR AND CEREBROVASCULAR EFFECTS OF LARYNGOSCOPY AND ENDOTRACHEAL INTUBATION IN NEONATAL PIGLETS, AND THE MODIFICATION OF THESE EFFECTS BY TOPICAL LIGNOCAINE

BY

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A THESIS

Submitted to the University of Cape Town for the Degree of Doctor of Medicine.

DECLARATION

"I do hereby declare that this submission is my own work and that to the best of my knowledge and belief it contains no material previously published or written by another person. There is also no material in this work that has previously been accepted for the award of any other degree or diploma from a university or institute of higher learning."

Signed.... Signed ..

Dated.....16/3/90.....

DEDICATION

This work is dedicated to my wife, Joanne, who shared in every aspect of this study, and who, despite the sacrifices that were asked of her, was a constant source of encouragement and support.

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1 THE EVOLUTION OF THE HYPOTHESIS

1.1 Introduction

1.1.1 Justification

1.1.1.1 Opening comments

"Perhaps no other organ of the body is less adapted to an experimental study of its circulation than the brain"

C.J.Wiggers.

These words were written in 1905 (Wiggers, 1905) and still hold true today. The undertaking of any study involving the accurate measurement of cerebral blood flow remains a formidable task, despite the technological advances of our time. The relevence of such work is of increasing importance, since interventional techniques are now available which may improve survival rates. The development of clinically applicable interventional procedures is largely due to clinical research advances and the availability of sophisticated diagnostic and support equipment.

Clinicians today are in a better position to anticipate potential difficulties, and have both the expertise and infrastructure to deal with many such situations. There are however, many frequently performed clinical procedures where possible detrimental effects have not been adequately investigated. All too often the final result is explained in terms of the original perceived pathology, ignoring the possibility that at least some part of the damage may have been due to the clinical procedure itself.

Endotracheal intubation of neonates is a frequently performed procedure in both the delivery room and neonatal intensive care areas. In the Groote Schuur Hospital group many such intubations are carried out by relatively junior staff under emergency conditions. The procedure itself is frequently complicated and prolonged, and may result in added trauma and damage to the neonate. For those babies that survive there are at present no available data that address long term adverse neurodevelopmental or behavioral sequelae.

Recently there have been a number of papers published (Friesen et al, 1987; Stow et al, 1988; Perlman and Volpe, 1983; Raju et al, 1980) dealing with the cerebrovascular effects of endotracheal intubation and endotracheal suctioning in neonates, with almost uniform conclusions on the intracerebral pressure changes induced by tracheal irritation. Bearing in mind that the sensory stimulus of endotracheal suctioning and endotracheal intubation are in

many ways comparable, it seems logical that the two procedures should have similar cerebrovascular effects. Considering that endotracheal intubation is often performed on neonates suffering from various degrees of asphyxia, a condition known to cause cerebral vasodilatation and increased cerebral blood flow (Lou et al, 1979), the potential for superadded injury exists.

There is a wealth of literature describing the potent hypertensive effects of laryngoscopy and endotracheal intubation in adult patients undergoing general anaesthesia. During my time as an anaesthetic registrar it appeared to me that these responses were largely unrecognised or ignored in neonates during resuscitative procedures. Endotracheal intubation in the presence of hypoxia and concurrent hypercarbia, when cerebral autoregulation is less effective (Lou et al, 1979) and sympathetic stimulation maximal, may well cause untoward intracerebral changes in these newborn infants.

A comprehensive literature search reveals that very little detailed work has been published in this field. This study has evolved from a desire to evaluate the detailed cardiovascular and cerebrovascular effects of laryngoscopy and endotracheal intubation in neonates, and having defined the response, to attempt to modify those aspects of the response deemed to be deleterious.

1.1.1.2 The Hypotheses

The 2 hypotheses to be tested by these studies were as follows:

1. That the procedure of prolonged laryngoscopy and/or endotracheal intubation causes significant adverse changes in the cardiovascular and cerebrovascular systems of the spontaneously breathing, hypercarbic, hyperoxic newborn piglet,

2. That such changes caused by laryngoscopy and/or endotracheal intubation may be advantageously modified by the application of a local anaesthetic agent (lignocaine) to the laryngopharyngeal and tracheobrochial areas prior to the stimulation.

1.1.1.3 Laryngoscopy and Endotracheal Intubation - History

Tracheal insufflation in animals was described by Vesalius in 1555 (Wedley, 1979), and by Robert Hooke in 1667 (Hooke, 1667). Kite described oral and nasal intubation for resuscitation of the apparently drowned in 1788 (Davison, 1951), and in 1880 MacEwen published a technique of passing a tube, in the conscious patient, from the mouth into the trachea using his fingers as a guide (MacEwen, 1880). These early attempts were to prevent aspiration pneumonia following surgery of the upper air passages. In the days prior to the use of muscle relaxant drugs, blind nasal intubation pioneered by Rowbotham (Rowbotham, 1920) was

popular as it was usually quicker than direct-vision oral intubation. With the introduction of muscle relaxants this technique gave way to oral intubation under direct vision, (rather than intubation under deep anaesthesia), and was popularised by Bourne (Bourne, 1947).

1.1.1.4 Literature review

1.1.1.4.1 Cardiovascular effects

1.1.1.4.1.1 Overview of available data

####### 1.1.1.4.1.1.1 Introduction

The pathophysiological complications of laryngoscopy and endotracheal intubation are no less important than its more obvious side-effects of trauma and mechanical damage to soft tissues. There are widespread effects following such stimulation, with some changes remaining undetected when only routine monitoring methods are used. Whilst such perturbations may be of little significance in healthy adults, the potential for widespread damage is known to exist where there is underlying disease such as essential hypertension (Prys-Roberts et al, 1971). These damaging effects may well be worsened by combinations of abnormalities such as hypercarbia, acidosis and

hypoxia that are frequently noted in distressed neonates. Much of the motivation for this study stems from the author's belief that the global response excited by this insult has not been adequately investigated in the newborn, in whom major physiological adaptations of virtually all systems are underway at the time of birth. The transition from placental to lung respiratory exchange, the change from a cardiovascular system in series to one in parallel, the need for an autonomous thermoregulatory centre and many other changes immediately distinguish the neonate from the adult and underscores the need for investigation aimed not only at defining the response, but also at identifying the differences peculiar to this group of patients.

Almost all of the work published on the effects of laryngoscopy and intubation concerns adult responses, and very few references to neonates are to be found in the current English literature. The direct extrapolation of adult responses to predicted neonatal behaviour is scientifically untenable and in many cases has been shown to be inaccurate. Data from neonatal studies is essential. The following review will perforce concentrate on the known changes (mostly adult) and will serve as a background from which to draw comparisons to the neonatal (piglet) data presented in this thesis.

1.1.1.4.1.1.2 Pressure/time intervals

1.1.1.4.1.1.2.1 Arterial pressure

1.1.1.4.1.1.2.1.1 Adult

The first detailed reports of the effects of laryngoscopy and intubation came from workers studying the electrocardiographic effects of the stimulus. Observations of acute cardiac dysrhythmias (Reid and Brace, 1940) were followed by numerous other reports of cardiac disturbances accompanying endotracheal intubation. Further studies reported on the marked increase in heart rate and arterial blood pressure associated with laryngoscopy and intubation (Burstein, LoPinto and Newman, 1950; Burstein, Woloshin and Newman, 1950; King et al, 1951; Noble and Derrick, 1959; Wycoff 1960; Takeshima, Noda and Higaki, 1964; Gibbs 1967; Forbes and Dally, 1970; Prys-Roberts et al, 1971). The average elevation in mean systolic and diastolic pressures after laryngoscopy and endotracheal intubation were more than 53 and 34 mmHg respectively (King et al, 1951). Other studies (Takeshima Noda and Higaki, 1964; Forbes and Dally, 1970) indicated that the mean increase in the arterial pressure following laryngoscopy and intubation was of the order of 20-25 mmHg, with maximal changes of about 40-45 mmHg in a few subjects. Derbyshire, Smith and Achola (1987) demonstrated mean increases of around 25-30 mmHg after laryngoscopy and intubation in 30 healthy women undergoing elective gynaecological surgery.

Kautto and Heinonen (1982) studied 48 healthy adults undergoing elective orthopaedic and oto-laryngological surgery, and in the control group of 16 patients found a mean systolic pressure increase of about 40 mmHg, and a diastolic pressure increase of about 30 mmHg accompanying the laryngoscopy/intubation. Laurito and colleagues (1988) reported very similar results in 10 patients (systolic pressure increase = 40 mmHg, diastolic pressure increase = 33 mmHg, and mean arterial pressure increase = 40 mmHg). In all of these studies laryngoscopy was performed for at least 45 seconds before the endotracheal tube was inserted.

Dohi and colleagues (1982) reported systolic and diastolic pressure increases of 76 mmHg and 39 mmHg respectively after endotracheal intubation preceded by only 30 seconds of laryngoscopy.

Dohi and Gold (1979) reported mean arterial pressure increases of 15 mmHg (16%) (95 -110 mmHg) following laryngoscopy and intubation in 19 healthy adult males.

Abou-Madi, Kezler and Yacoub (1975) noted a 67 mmHg systolic pressure and a 37 mmHg diastolic pressure increase at one minute post laryngoscopy/intubation. No details on the duration of laryngoscopy are given in this report, but the systolic and diastolic pressure increases during the laryngoscopy were 31 mmHg and 18 mmHg respectively.

Siedlecki (1975) reported on the effects of laryngoscopy and laryngotracheal spraying with lignocaine prior to intubation in both anaesthetised and conscious patients. In the anaesthetised

patients there was a 31% increase in mean arterial pressure during laryngoscopy and spray, and a 29% increase after intubation. In all of the above cases the patients were anaesthetised and paralysed, having undergone various combinations of induction techniques.

There are very few reports the effects of laryngoscopy and intubation in unanaesthetised or unsedated adults. Siedlecki (1975) performed laryngoscopy and intubation on 12 unsedated patients who received only local anaesthetic spray to the respiratory mucosa. In these conscious patients laryngoscopy and spray caused a 55% increase in mean arterial pressure and was followed by a 39% increase in pressure after intubation. The pressure response was reported to have lasted 12 minutes.

The collective data presented above do not constitute a homogenous group. Any conclusions drawn should bear this in mind. In most cases the agent used in the induction of the anaesthetic had a cardiodepressant effect and thus any blood pressure changes resulting from the procedures could conceivably be less than would be expected in the conscious unmedicated subject.

Long, Zebrowski and Graney (1982) compared the cardiovascular effects of laryngoscopy and intubation in anaesthetised and awake/sedated patients. They showed highly significant increases in mean arterial pressure in both groups within 0.5 minutes following intubation, and these changes persisted for longer than 3 minutes. The mean arterial pressure increases were 31 mmHg in

the anaesthetised group, and 14 mmHg in the awake patients. These data suggest that general anaesthetic and muscle relaxation in some way increase the hypertensive response to laryngoscopy/intubation. The possibility that the anaesthetic agents increase the sensitivity of the vasculature to noxious stimuli may explain this finding.

There is no data presented in this study that specifically relates to the laryngoscopy, nor are there details of the time taken for the pressure to return to the baseline value.

Bedford and Marshall (1981) showed mean arterial pressure increases after laryngoscopy and intubation ranging from 21% - 42% for 4 different combinations of anaesthetic agents, confirming the findings of others mentioned above.

Prys-Roberts and colleagues (1971) emphasised that hypertensive patients, treated or not, are prone to much greater fluctuations in arterial pressure than normotensive patients of the same age. In their study of treated and untreated hypertensive patients they showed increases in systolic pressure of up to 92 mmHg following laryngoscopy and intubation. Previous drug therapy modifies the response, but changes occur even when the individual is apparently well controlled on anti-hypertensive therapy. Increases of mean arterial pressure exceeding 100 mmHg have been described in patients (Forbes and Dally, 1970).

Dingle (1966) in a series of 19 hypertensive patients found a mean increase in systolic arterial pressure of 35 mmHg. Stoelting (1977) studied 36 patients with known cardiac disease during

non-cardiac surgery, and showed that in the control group of 12 patients who did not have prior viscous or intravenous lidocaine, there was an increase of 37 mmHg after laryngoscopy/intubation.

It is unlikely that treated or untreated hypertension of this degree will be seen in neonates, but the above information has been included to emphasise the degree of response possible by a reactive cardiovascular system.

1.1.1.4.1.1.2.1.2 Neonatal

There are very few references in the English literature on the effects of laryngoscopy and intubation in either human or animal term neonates. Whatever studies are available have usually been carried out on preterm newborns and often with non-invasive intermittent techniques. A greater literature exists regarding endotracheal suctioning and endotracheal fluid instillation, but this again concerns mainly the ventilated preterm infant, often with respiratory distress syndrome.

Charlton and Greenhough (1988) investigated blood pressure and heart rate responses to tracheal intubation and concluded that there was no hypertensive response to the awake intubation of unanaesthetised term neonates. They did however show significant increases in systolic and diastolic pressure in term neonates intubated following halothane induction of anaesthesia. Pre-term

infants at the same post-conceptional age also showed similar significant increases in blood pressure following intubation. The authors have not separated the effects of laryngoscopy from those of the laryngoscopy/intubation, nor have they presented any data on the method or timing of the laryngoscopy. Blood pressure and pulse rate were measured using 2 different non-invasive intermittent blood pressure monitoring devices, with no indication given as to which machine was used on which patient. The timing of the baseline pressure measurements was different for the anaesthetised and awake groups and only a single measurement was taken "as the tube entered the trachea". There was no further mention made of blood pressure changes in the ensuing minutes following the intubation. It is difficult to draw any definite conclusion based on the findings presented in this paper.

Although there has been recent interest (Berry and Gregory, 1987) in the potential danger of intubating neonates with special reference to intraventricular brain haemorrhages, neither of these articles has cited references that present blood pressure values.

Friesen, Honda and Thieme (1987) studied the effects of intubation in preterm neonates, with special reference to changes in anterior fontanel pressure, systolic and mean arterial pressure and heart rate. The patients were divided into 2 groups. In group 1, 0.02mg/kg of atropine was given

intravenously and awake intubation was performed. Group 2 patients received 0.02 mg/kg atropine and 0.01mg/kg pancuronium intravenously, and one of 4 anaesthetics - 0.75% isoflurane, 0.5% halothane, 20 microgram/kg fentanyl ivi, or 2 mg/kg ketamine. This was followed by 10 minutes of mask anaesthetic ventilation to establish baseline levels, and then laryngoscopy and intubation were performed. In group 1 (the awake group) there was a 20% increase (63 +/-7 mmHg to 74 +/-11 mmHg) in systolic blood pressure ($p<0.05$) and a 15% increase (50 +/- 8 to 58 +/- 7 mmHg) in mean arterial pressure following intubation. In group 2 there was a 10% increase in both systolic (64 +/- 13 mmHg to 70 +/- 16 mmHg) and mean (50 +/- 12 mmHg to 55 +/- 18 mmHg) arterial pressures, with neither of these increases achieving significance. As with the previous study, blood pressure was measured using an intermittent non-invasive technique with recordings being made every minute. There is no mention of blood pressure readings taken during laryngoscopy or in the 5 minutes after the intubation. Of more significance is the lack of data on blood gas values before, during and after the laryngoscopy/intubation. Considering the rapidity with which the blood gas picture can change in these patients, and the profound effects on cardiovascular dynamics that such changes may have, this information is essential to allow realistic deductions from this data.

In a previous article on the cardiovascular effects of various anaesthetic techniques in preterm neonates, Friesen and Henry (1986) demonstrated only a 9% increase in systolic blood pressure after intubation, and on the basis of these two studies Friesen, Honda and Theime (1987) state that wide fluctuations in systolic pressure are probably not common following awake intubation in neonates.

Marshall and colleagues (1984) studied the physiologic changes associated with endotracheal intubation in 10 preterm infants. Six of these babies had respiratory distress syndrome with a blood gas picture of respiratory acidosis (pH < 7.25, PaCO2 > 45 mmHg). The remaining 4 neonates were intubated because of repeated apnoeic episodes associated with hypoxaemia. The mean age of the babies was 8 days (range 10hours to 30 days) and the mean gestational age was 29.4 weeks (range 26 - 37 weeks). The mean weight of these babies was 1.19 kg (range 0.55 - 2.63 kg). There is no mention made of the time taken with the laryngoscopy. The arterial pressure was continuously recorded via an umbilical or radial artery catheter. There were no anaesthetic agents given prior to the laryngoscopy/intubation and in all cases the babies were ventilated with 100% oxygen via a mask for "several" breaths.

Laryngoscopy and endotracheal intubation was associated with a significant increase in systolic blood pressure from 65 +/- 20 mmHg to 95 +/- 36 mmHg (46%). The systolic blood pressure returned to the baseline within a mean time of 56 +/- 69 seconds (range 8 - 230 seconds).

The systolic blood pressure after intubation was not significantly increased when compared with the the maximum systolic pressure after laryngoscopy (84 +/- 24 mmHg). The post intubation pulse pressure, when compared with the baseline pulse pressure increased significantly ($p<0.01$) with a mean change in pulse pressure of 13 +/- 9 mmHg. Accidental intubation of the oesophagus during the intubation attempts was associated with an increase in systolic pressure of 28 +/- 4 mmHg in 2 babies.

The authors of this study commented that there was substantial individual variation in the blood pressure during the endotracheal intubation. There was no correlation between the maximum systolic blood pressure and the age of the baby, the time required for the intubation, or the number of intubation attempts. Laryngoscopy was however always associated with increases in the systolic, mean and diastolic blood pressures.

Marshall and coworkers (1984) also showed that the transcutaneous oxygen tension decreased from 83 +/- 14 mmHg before the laryngoscopy/intubation to 49 +/- 28 mmHg after the event.

The hypertensive response is reported to have occurred before the onset of hypoxaemia in this study. This temporal relationship is taken as evidence in support of the theory that the hypertensive response is on the basis of increased nervous activity in the

cervical sympathetic efferent fibres resulting from mechanical stimulation of the epi- and laryngopharynx (Tomori and Widdicombe, 1969). This may be associated with increased sympathoadrenal activity, as hypothesised as the cause of the hypertension seen with laryngoscopy in anaesthetised adults (King et al, 1951). Given the rapid response of the cardiovascular system to the insult, Marshall et al (1984) feel that the hypertension is more likely to be a neural or humoral response than an effect of hypoxia or hypercarbia. As in the previous study, there is no data presented as regards the detailed blood gas changes associated with the intervention. Given the fact that 6 of the 10 infants were acidotic to begin with, and that following laryngoscopy and intubation the neonates were certainly hypoxic, the assumption that hypoxia and hypercarbia were not responsible for the cardiovascular perturbations cannot be made. In addition to the neural and humoral factors involved, because these babies were intubated awake, a certain proportion of the blood pressure elevation may have resulted from the struggling of the baby during the procedure.

Hinkle (1983) studied the effects of laryngoscopy in 5 awake neonates of unspecified age. All had been preoxygenated with 100% oxygen and premedicated with 0.1mg atropine ivi, prior to the procedure. When subjected to 30 seconds of continuous laryngoscopy, all 5 neonates showed a decrease in systolic blood pressure (mean decrease of 20 +/- 6 mmHg) associated with a decrease in transcutaneous oxygen tension (mean decrease of 83

+/- 20 mmHg). Within 5 minutes all parameters returned to baseline levels. When the same neonates were subjected to a second laryngoscopy, but this time under continuous oxygenation with a Miller 0 Foregger oxyscope blade after 2 minutes of preoxygenation, there were insignificant decreases in blood pressure (mean decrease of 0.8 +/- 6 mmHg) and transcutaneous oxygen tension (mean decrease of 30 +/- 6 mmHg) when compared to the previous laryngoscopy.

The different responses to essentially the same stimulation in these two studies is difficult to explain. There are however some distinct differences in the sample groups. In the Hinkle (1983) study the mean weight of the babies was 2.66 kg as compared to 1.19 in the Marshall et al (1984) study. In addition, Hinkle (1983) does not mention the age of the neonates involved in his trial, but one must assume that they were closer to term (on the basis of body weight) than in the Marshall study. Of greatest significance is that the babies in the Marshall (1984) study had lower baseline transcutaneous oxygen tensions and almost certainly significantly lowered respiratory reserve, than the babies in the Hinkle (1983) study. The decrease in blood pressure in the Hinkle study is difficult to explain and additional information as regards the acid/base balance in the two groups is essential before any meaningful explanation can be attempted.

Lindgren and Saarnivaara (1985) reported on the cardiovascular responses to laryngoscopy and intubation following an inhalational halothane induction in 27 children with a mean age

of 1.5 +/- 0.6 years. Systolic blood pressure increased by 10.8 +/- 1.9 mmHg ($p<0.001$), and diastolic pressure by 6.0 +/- 1.4 mmHg (mean +/- standard error of the mean). The increase in the diastolic pressure was insignificant and indicates a widening of the pulse pressure following laryngoscopy/intubation. This response was noted to be less than that seen following intubation in children anaesthetised with thiopentone and suxamethonium (Lindgren, Saarnivaara and Himberg, 1980). Of interest was the fact that 5 minutes after the intubation the systolic and diastolic pressures remained unchanged from those immediately after the intubation; no data after 5 minutes post intubation is provided.

Stow et al (1988) reported on the cardiovascular responses to laryngoscopy and endotracheal intubation in 14 awake and 10 anaesthetised neonates. Demographically the two groups were similar for age (awake group = 41 +/- 1 weeks; anaesthetised group = 43 +/- 1 weeks) but not for weight (3.2+/-0.2Kg vs 3.9+/-0.2Kg). They found that although there were increases in systolic blood pressure (greater increases seen in the awake group) none of the changes were statistically significant because of the small sample sizes. The trend was definitely to an increase in systolic pressure (mean +/- standard deviation) following the insult (no separate figures for laryngoscopy given) with a change from 94 +/- 4 mmHg to 120 +/- 14 mmHg in the awake group, and from 92 +/- 3 mmHg to 104 +/- 8 mmHg in the anaesthetised and paralysed group. The neonates in the awake

group did not have any premedication other than atropine 0.02mg/kg given iv 2 minutes before laryngoscopy. The anaesthetic group were given a standard thiopentone/atropine/ suxamethonium induction.

As regards nasotracheal intubation in neonates, the single reference reviewed (Kelly and Finer, 1984) reported on 30 neonates with birth weights from 580 to 3450 grams (25 to 40 weeks gestation) who were prospectively studied during nasotracheal intubation. The infants were randomized to receive atropine 0.01mg/Kg, atropine 0.01mg/kg plus pancuronium 0.1mg/kg, or no medication (controls) prior to intubation. There was a significant increase in the mean blood pressure in all babies during the laryngoscopy and intubation, with the greatest change occurring in the control group (mean increase of 22 mmHg). These changes represented an average increase in mean arterial pressure for all three groups of 57% and remained at least 20% above the baseline level for an average of 25.5 seconds. The response in this study is difficult to interpret since it was shown by Tomori and Widdicombe (1969) that the most potent area of stimulation of the respiratory tract for the generation of a cardiovascular response is the nasopharynx. How much of the response seen in this study was generated by irritation of the nasal mucosa by the tube, both during insertion and thereafter, is impossible to assess. In addition there was a significant decrease in transcutaneous oxygen tension in all three groups, with a mean decrease of 27 mmHg.

The above presented data confirm the impression that laryngoscopy and intubation increase blood pressure, but there are few data or studies that examine the response in a detailed temporal manner.

1.1.1.4.1.1.2.1.3 Animal

Animal studies on the cardiovascular effects of irritation of the respiratory tree are best represented by the work of Tomori and Widdicombe (1969). In their series of experiments they stimulated various areas of the respiratory mucosa with a nylon fibre and measured the electrophysiological and other responses.
The systemic arterial blood pressure response to stimulation at different sites in the respiratory tracts of 8 paralysed, ventilated adult cats indicated that the largest reflex increase in blood pressure was evoked from the epipharyngeal region (22 +/- 4.9 mmHg) and the smallest from the tracheobronchial region (10.0 +/- 0.8 mmHg).
In 8 non-paralysed, spontaneously breathing cats the changes in pressure were deemed to represent a combination of reflex and mechanical effects. There were clear decreases in diastolic and mean blood pressures during and after the strong ventilatory efforts evoked by all the mechanical stimulations. There were even greater increases in both systolic and mean blood pressures

during the strong expiratory efforts from coughing elicited by stimulation of the tracheobronchial and laryngopharyngeal mucosa. The initial reaction in most cases was a substantial increase in systolic and mean pressure during the stimulation period. These increases were however masked on many occasions by large respiratory variations in blood pressure (aspiration reflex). The greatest systolic pressure increases were elicited from the laryngopharyngeal area (47 +/- 3.8 mmHg) while the largest mean arterial pressure increases came from the tracheo-bronchial area (19 +/- 3.2 mmHg). The most significant mean pressure decreases were seen in the epipharyngeal area (-23 +/- 3.2 mmHg).

Significant increases in both systolic and mean blood pressures were noted during the strong expiratory efforts of coughing elicited from tracheobronchial and laryngopharyngeal stimulation. These increases were most prominant at the beginning of or during the period of irritation. These results indicate that the primary reaction to laryngeal stimulation is a hypertensive one and that this hypertension is associated with an increase in sympathetic efferent fibre discharge. The fact that an increase in sympathetic activity was seen in paralysed animals makes mechanical effects and reflex respiratory movements an unlikely cause for the increased impulse traffic in the cervical sympathetic efferent fibres, and raises the possibility that the hypertension could be partly due to release of catecholamines from the adrenal medulla. This aspect is discussed in more detail below.

The most striking difference in the results of this study and the clinical studies reported above, is the absence of the ventilatory interference in the human patients due to the anaesthetic agents used. This removes the mechanical effects leaving a more accurate measure of the direct vasoconstrictor hypertension.

1.1.1.4.1.1.2.2 Time intervals

1.1.1.4.1.1.2.2.1 Adults

With the introduction of direct and continuous arterial blood pressure monitoring methods it was noted that changes occurred as soon as laryngeal stimulation commenced, before the intubation was performed (King 1951). This work was carried out on lightly anaesthetised healthy adult patients. The systolic and diastolic pressures both rose within 5 seconds of laryngoscopy, reached a peak within 1-2 minutes, and returned to prelaryngoscopy levels within 5 minutes. Stoelting (1977) demonstrated that near maximal pressor responses were present after 45 seconds of laryngoscopy, with sigificantly raised mean arterial pressures seen after 15 seconds. Prolongation of the laryngoscopy to 60 seconds produced less than a 5mmHg additional increase in mean

arterial pressure above the value seen at 45 seconds. The mean arterial pressure was restored to baseline levels within 2 minutes of intubation in this study.

Stoelting (1978) reported on blood pressure changes in 12 patients (control group) who had "short duration " laryngoscopy (less than 15 seconds). Maximal increase in mean arterial pressure occurred 5-15 seconds after tracheal intubation, averaging 17 +/- 3 mmHg. Mean arterial pressure returned to baseline levels within 2 minutes of the start of the laryngoscopy. He concluded that "when tracheal intubation can be reliably accomplished with a short period of direct laryngoscopy, pharmacologic attempts to minimise the pressor response are not helpful".

In a study by Abou-Madi, Kezler and Yacoub (1975) on normotensive adults, blood pressure only returned to baseline levels after a 5-10 minute period. These time intervals were similar to those reported by Dohi and Gold (1979) where peak pressures were seen within 1 minute and baseline pressures attained within 5 minutes from the intubation.

Kautto and Heinonen (1982) demonstrated a maximal pressure response within 30 seconds of the start of laryngoscopy. In their study blood pressure was still elevated slightly above control levels at 3 minutes after the laryngoscopy. Unfortunately they did not give detail as to the time taken to return to baseline levels.

The fact that laryngoscopy and intubation induce reflex hypertension in adult humans is seen to be well demonstrated in the literature. The response is rapid with most reports showing maximal arterial pressures within one minute of start of laryngoscopy. The time taken for the blood pressure to return to the baseline level is still not well defined, but in most studies the baseline levels were attained within 5 minutes of the stimulation.

1.1.1.4.1.1.2.2.2 Neonates

The data available for neonates is sparse and there is very little information specifically concerning the time-to-peak and time-to-baseline intervals. In the study by Friesen, Honda and Thieme (1987) the duration of laryngoscopy was 5 - 30 seconds in both the awake intubation and the anaesthetised groups, with a mean time of 22 seconds, but because of the intermittant method of pressure measurement, information as to the pressure/time relationship is not possible. Charlton and Greenhough (1988) make no mention of the duration of laryngoscopy at all, and nor do Lindgren and Saarnivaara (1985).

In the study on nasotracheal intubation (Kelly and Finer, 1984) the increase in blood pressure began with the insertion of the nasotracheal tube, and remained elevated for an average of 25.5 seconds. The average duration of the intubation, from insertion

of the tube into the nose until it was placed in the trachea was 32 +/- 13 seconds, and the time from insertion of the laryngoscope into the mouth to passage of the tube through the cords was 26 +/- 10 seconds.

1.1.1.4.1.1.2.2.3 Animals

No mention is made of the time-to-peak blood pressure or of the time-to-baseline pressure in the study by Tomori and Widdicombe (1969).

1.1.1.4.1.1.2.3 Laryngoscopy vs L/I

Prys-Roberts (1971) stated that "the majority of patients studied by us produced a reflex tachycardia and hypertension well before the act of intubation, though the effect was often enhanced by either spraying the larynx, or by intubation."

Stoelting (1977) reported on the effects of laryngoscopy alone and the combination of laryngoscopy and intubation in patients given intravenous lidocaine just before laryngoscopy. He found that endotracheal intubation had a superadded effect to that of laryngoscopy, and that mean arterial pressure increased an additional 22mmHg with the placement of the endotracheal tube. This effect was only recognizable when laryngoscopy was prolonged

over 30 seconds because the increase in mean arterial pressure is not maximal until 30 - 45 seconds of laryngoscopy. After 45 seconds it becomes possible to separate the relative contributions of the laryngoscopy and the endotracheal intubation to the ultimate blood pressure increase.

In a study by Denlinger, Ellison and Ominsky (1974) the response to an initial laryngoscopy and spraying of the cords with saline was greatly exaggerated when laryngoscopy and intubation was performed. This suggests that tracheal intubation is accompanied by sympathoadrenal (or other) stimulation additional to that of laryngoscopy alone.

Shribman, Smith and Achola (1987) demonstrated that laryngoscopy alone generates essentially the same pressor response and sympathoadrenal response as does laryngoscopy followed by intubation. Laryngoscopy with intubation, however, was associated with significant increases in heart rate which were not seen in the laryngoscopy-only group. They had difficulty in explaining this finding, but felt that perhaps laryngoscopy produced a balanced stimulation of vagal and cardiac accelerator fibres, whereas intubation produced less vagal stimulation.

Laryngoscopy alone appears to stimulate a response similar to and on occasion equal to that of laryngoscopy and intubation. There are few data available comparing the haemodynamic responses to

blind nasal/oral intubation, and laryngoscopy and intubation which would allow an assessment of the contribution of the laryngoscopy to the pressor response.

The only reference to the cardiovascular response to nasotracheal intubation uncovered was that of Prys-Roberts and coworkers (1971). In this study 4 patients undergoing dental procedures were monitored. Nasotracheal intubation was performed after inhalation of 10% carbon dioxide and there were no significant changes noted in systolic and mean arterial pressure, or in heart rate. In two of these patients, previous laryngoscopy had produced a marked hypertensive response.

Published data thus indicate that endotracheal intubation usually adds an additional pressor element to the cardiovascular response, but that this response is mainly the result of pressure on the laryngeal tissues during the laryngoscopy.

1.1.1.4.1.1.2.4 Carinal stimulation

There appear to be differences in the responses to stimulation of various areas of the human respiratory tract (Dohi and Gold, 1979; Corbett, Kerr and Prys-Roberts,1969). Dohi and coworkers (1982) studied the effects of carinal stimulation in paralysed intubated patients. In the control group (no form of neural blockade) bronchocarinal stimulation caused significant increases in blood pressure and heart rate. The magnitude of the systolic blood pressure response ranged from 10 - 45 mmHg. The mean

systolic blood pressure change was however significantly less than that seen with laryngoscopy and intubation (128 +/- 17 to 157 +/- 21 mmHg with carinal stimulation as opposed to 109 +/- 15 to 185 +/- 34 mmHg following laryngoscopy/intubation).

Tomori and Widdicombe (1969) also showed significant increases in blood pressure following stimulation in the trachea-bronchi area of the respiratory tract. In paralysed ventilated cats there was a mean arterial blood pressure increase of 10 +/- 0.8 mmHg during the stimulation, and in anaesthetised but spontaneously breathing cats the mean arterial pressure increase was 19 +/- 3.2 mmHg. Of note is the difference in the magnitude of the response which suggests that in the non paralysed group there is a significant contribution to the pressor response from the protective respiratory reflexes.

Dohi and Gold (1979) showed that in addition to pressor responses, carinal irritation in 19 anaesthetised, paralysed patients, also led to a 27% increase in pulmonary resistance and a 10% decrease in pulmonary compliance. These changes had reverted to baseline levels within 5 minutes of the stimulus.

1.1.1.4.1.1.3 Heart rate changes

1.1.1.4.1.1.3.1 Adult

Manipulation in and around the larynx has occasionally been found to cause cardiac arrhythmias and arrest (Strong, 1974), and stimulation of the superior laryngeal nerve in experimental animals has been shown to induce similar dysrhythmias (Suzuki, 1967).

Most authors have found increases in heart rate following laryngoscopy/intubation in adults, but reports in the literature vary as to the extent of the heart rate increase ranging from 11-48 % (see table C2-1).

In one of the earliest studies, Burstein, LoPinto and Newman (1950) reported a tachycardic response to laryngoscopy and intubation with post insult heart rates ranging from 110 to 170 beats per minute recorded from 43 of 109 patients.

Derbyshire, Smith and Achola (1987) studied healthy women undergoing minor gynaecological surgery. They noted an increase in heart rate from 89 to 105 beats per minute (18%), with a maximum rate at 1 minute, and a return to the baseline value by 5 minutes.

Forbes and Dally (1970) also looked at a group of women undergoing minor gynaecological surgery and showed a similar increase in heart rate (10%). In this study the maximum heart rate was achieved within 1 minute, and although a

time-to-baseline (ie time required for the heart rate to return to the baseline level) was not given, the heart rate was still elevated at 3 minutes post insult.

Dohi and coworkers (1982) showed in a control group of 12 patients, an increase in heart rate from 80 to 116 beats per minute (45%) following laryngoscopy/intubation.

Long, Zebrowski and Graney (1982) reported an increase in the heart rate of a control group of 8 anaesthetised patients after laryngoscopy/intubation. The rate changed from 92 to 123 beats per minute (34%) at the time of intubation and had receeded toward the baseline value (but was still elevated) at 3 minutes post intubation.

Laurito and colleagues (1988) carried out a well designed and controlled trial comparing the effects of various combinations of agents in the damping of the cardiovascular responses to laryngoscopy/intubation (45 seconds). In the control group there was a maximum increase in heart rate of 48% following the intervention. Unfortunately no time intervals were given.

There is closer consensus as to the direction of the change and there were no series that demonstrated a marked bradycardic response to the laryngoscopy/intubation in adults. Forbes and Dally (1970) did however show that of 22 patients there were 7 patients with decreased heart rates during laryngoscopy, but in no case did the heart rate decrease below 70 per minute.

The change in heart rate after laryngoscopy and intubation in hypertensive patients was studied by Prys-Robets and colleagues (1971), and they reported increases of between 36% and 37% in treated and untreated groups respectively.

As in the review of the blood presssure changes it must be emphasised that in all of these studies the patients had been given some form of anaesthetic, usually a thiopentone induction following an opioid premedication, succinyl-choline muscle relaxation, and post intubation ventilation with 70% N2O and 30% oxygen.

Table C2-2 lists data from some of the reports reviewed. Values are taken from tables or estimated from figures in those cases where numerical data is not tabulated. In those comparitive studies from which data is quoted the control group values are given.

Table C2-2: Table of reports showing heart rate changes following laryngoscopy and intubation.

First author	B/line	Post L	Post I	%	TTP	TTB
	(beats per minute) (mean +/- standard deviation)				(seconds)	(minutes)
Stoelting (1977)	72+/-4	83+/-5	99+/-4		-	3
Abou-Madi (1975)	72+/-9	81+/-11	100+/-11		20	5-10
Kautto (1982)	93+/-5	-	113+/-5		30	>3
Derbyshire (1987)	97+/-5	-	105+/-4	18%	60	5
Long (1982)	82+/-12	-	95+/-19		30	3
Dohi (1979)	80+/-15	-	90+/-10	11%	-	-
Dohi (1982)	80+/-11	-	116+/-11	45%	-	5-6
Forbes (1970)	88+/-4	-	112+/-4	10	120	>5
Laurito (1988)	79+/-4	-	117+/-3	48%	-	<6
Hamil (1981)	87+/-5	97+/-5	108+/-3	11%	30	2

Leslie (1987)	74+/-6	-	99+/-6		-	<10
Chung (1988)	88+/-15	-	117+/-20	33%	-	<5
Bedford (1981)	86+/-7	-	102+/-7	24%	<1	-
	83+/-5	-	105+/-5	27%	<1	-

B/line = baseline heart rate, Post L = post laryngoscopy, Post I = post intubation, % = percentage change, TTP = time taken to attain the peak heart rate, TTB = time taken to return to the baseline heart rate.

1.1.1.4.1.1.3.2 Neonatal

There is very little specific data on the cardiovascular effects of laryngoscopy and intubation in neonates. What work has been published is often retrospective and in many cases difficult to interpret because of underlying pathology, differing anaesthetic techniques and drugs, innaccurate monitoring techniques and insufficient study periods. Endotracheal intubation in neonates has usually been reported as being associated with a bradycardia (Marshall, 1984; Mirakhur, 1981; Hinkle, 1983). In most of the published work that report blood

gas status, there are significant decreases in arterial oxygenation coincident with the laryngoscopy and intubation. Bearing in mind the sensitivity of the neonate to arterial desaturation and the fact that in many of the published cases there is already limited cardiorespiratory reserve, it is very likely that the hypoxia plays some role in the cardiovascular response. Mirakhur (1981) reported that the fall in heart rate was almost instantaneous following laryngoscopy and cord spraying, but that in all cases there was an increase in rate as soon as intubation was carried out. In his study in children ranging from 1 to 14 years, there are no data for arterial oxygenation reported.

Hinkle (1983) demonstrated that significant decreases in heart rate following laryngoscopy were only present when the laryngoscopy was associated with hypoxia. Two separate periods of laryngoscopy were performed on 5 healthy neonates (no ages given). All babies were given 0.1mg of atropine intravenously prior to the study.

In the first study period the neonates were given 2 minutes of pre-oxygenation with 100% oxygen. Following this they were subjected to 30 seconds of laryngoscopy, during which time blood pressure, heart rate and transcutaneous oxygen saturation was non-invasively monitored. All neonates showed a significant decrease (23 +/- 7 beats per minute) in heart rate during the 30 second of stimulation. This reduction in rate was accompanied by a significant lowering of the transcutaneous oxygen tension (mean decrease of 83 +/- 20 mmHg).

In the second study period, 5 minutes after the first, following return to baseline of all parameters, preoxygenation with 100% oxygen was again performed. Laryngoscopy was then undertaken with a Miller O Foregger oxyscope blade delivering 100% oxygen at a flow rate of 3.5 litres per minute. All patients were intubated within 45 seconds on this second attempt, and the same parameters were measured as in the previous stimulation period. There were insignificant changes in the heart rate and transcutaneous oxygen tension when compared with those following the first insult. Heart rate decreased by a mean of 0.8 +/- 7 beats per minute and the transcutaneous oxygen tension dropped by a mean of 30 +/- 6 mmHg.

This study lends support to the theory that the bradycardia often seen with prolonged intubation attempts is a hypoxia mediated event, rather than a result of vagal stimulation (Mirakhur, 1982). No time intervals are reported with this study, but personal experience has shown that when bradycardia is encountered during intubation it is usually following a protracted period of laryngoscopy, and not immediately after the initiation of the stimulus. Charlton and Greenhough (1988) studied the effects of laryngoscopy and intubation in 45 term neonates and 15 infants. The term neonates were defined as babies aged 28 days or less born after 36 weeks of gestation. The infants were 4 weeks or older post natal, but of the 15 there were 12 born at 33 weeks or less. The babies were divided into 3 different groups depending on the type of anaesthetic administrered (awake intubation, halothane and nitrous oxide, or

thiopentone and a muscle relaxant-atracurium or pancuronium). The mean weights of the neonates did not differ significantly (range 3.05kg - 3.24 kg), but the infants (2.14 kg) were significantly lighter than the neonates. Blood pressure and heart rate were measured with non invasive methods and all babies were preoxygenated. The responses of the neonatal groups were analysed for differences while the infants were studied as a single group. In no case did laryngoscopy and intubation (no times mentioned) lead to decreases in heart rate and in all groups the stimulus was associated with an increase in the heart rate. In the "awake" neonates and in the infant group there were insignificant changes with increases of 11% (141 - 156 beats per minute) and 3% (171 - 176 beats per minute) respectively. The changes in the "halothane" and "thiopentone" groups of the neonates were significant, with increases of 7% (163 - 174 beats per minute) and 14% (140 -159 beats per minute) respectively. There are no blood gas data reported. Stow et al (1988) reported on heart rate changes in awake and anaesthetised neonates during laryngoscopy and intubation. Although there was a similar slight decrease in rate in both groups, there were no significant differences at all. Both groups of neonates had been given atropine before the procedure. The lack of data on blood gases make the interpretation difficult, and it may be that some of the changes noted were mediated by short periods of hypoxia to which the neonate is extremely sensitive.

Kelly and Finer (1984), while not providing detailed data or figures for heart rate changes, stated in their study on the effects of nasotracheal intubation in neonates that the stimulus was associated with significant decreases in heart rate and transcutaneous oxygen tension. This cardiovascular response was explained by the authors as being a parallel response to that seen in the newborn lamb with laryngeal chemoreceptor stimulation and to stimulation of the trigeminal diving reflex by cooling of the snout. Sudden hypoxia has been demonstrated to cause bradycardia in neonates subjected to tracheal suctioning (Simbruner et al, 1981). It may be that in many cases of bradycardia following intubation, the slowed heart rate is a result of the acute hypoxia associated with the insult, rather than from a direct reflex.

From the few data reported in the literature, there is no real consensus of opinion on the heart rate response to laryngoscopy and intubation in term neonates. It can be seen that what opinion there is at present, is based on anecdotal and uncontrolled reports.

Lindgren and Saarnivaara (1985) measured heart rate and ECG responses to laryngoscopy/intubation following a halothane induction in older children (mean age 1.5 +/- 0.6 years). Their findings indicated a significant increase in heart rate from 130

beats per minute to 140 beats per minute immediately following the laryngoscopy and intubation ($p<0.01$), with an additional increase in rate to 145 beats per minute at 5 minutes post procedure ($p<0.02$). There were no arrhythmias noted during the laryngoscopy or intubation in any of the children.

1.1.1.4.1.1.3.3 Animal

The animal data is even more sparse than for humans. Tomori and Widdicombe (1969) mention in passing that "there were increases in pulse pressure and heart rate during the hypertensive reactions from the four sites (nasopharyngeal, epipharyngeal, laryngeal and tracheal), the changes being especially conspicuous in the case of epipharyngeal and laryngeal stimulations". They do not tabulate or in any other way present any data on heart rate changes in their cats.

King and colleagues (1951) reported on the effects of laryngoscopy and intubation in 3 anaesthetised adult dogs. They comment that "the effects of laryngoscopy or tracheal intubation in the dog were uniform and were entirely different from those observed in man. There appeared to be marked vagal inhibition of the heart with slowing of the pulse rate and some fall in blood pressure". They were able to reproduce this effect, and were further able to reduce the effect and eventually abolish it by

giving successive doses of intravenous atropine or scopalamine. No data is given as to the acid-base balance in these animals during the experiment.

1.1.1.4.1.1.4 ECG changes

1.1.1.4.1.1.4.1 Adult

In an extensive electrocardiographic (ECG) study, Burstein, LoPinto and Newman (1950) demonstrated ECG abnormalities in 73 of 109 cases (68%) at the moment of intubation. In 43 of these patients the only ECG change noted was that of tachycardia. There were however a number of other arrhythmias reported at the time of intubation (not during the laryngoscopy): ventricular ectopic beats with bigeminal or trigeminal rhythm (10 cases), nodal rhythm (5 cases), sinus bradycardia in 4 cases, 7 cases of T-wave abnormality, 3 cases of marked increase in the PR interval, 2 cases if sinus arhythmia, 2 cases of ventricular tachycardia and one case of atrial fibrillation. It is difficult to interpret these results since the endotracheal intubation was performed at different stages of anaesthesia, and with different degrees of stimulation and different premedication drugs. Prolonged exploration and excessive instrumentation with repeated attempts at intubation, hypoxic episodes, respiratory obstruction before intubation, and tracheal irritation after intubation are

reported as aggravating the ECG disturbances. The ECG abnormalities noted in this series were short lived, lasting from 15 seconds to 10 minutes, and were "without grave consequence". The interpretation of ECG records taken over the time of laryngoscopy and intubation has been varied (Burstein, LoPinto, and Newman, 1950; Reid and Brace, 1940). Reid and Brace (1940) concluded that cardiac reflexes could originate in the trachea, larynx, bronchi or lungs and effect a response by a sudden increase in vagal tone. Both the efferent and afferent paths of the reflex were thought to be the vagus nerve, and thus the reflexes were called "vasovagal". Other workers, using a similar study design, hypothesised that all of the ECG changes could be attributed to stimulation of the cardio-accelerator nerves and increased sympathetic tone (Burstein, LoPinto and Newman, 1950; Burstein, Woloshin and Newman, 1950).

Takeshima, Noda and Higaki (1964) reported that almost all patients in their study showed some change in the ECG over the time of induction and tracheal intubation:

(i) P wave - There were no changes in P-R interval although in 9 patients (18%) there were changes in the height of the P waves directly related to intubation.

(ii) QRS intervals - There were no changes in QRS interval that could be related to intubation.

(iii) QT interval - In 3 patients (6%) there was a prolongation of the QT interval that began after the endotracheal intubation. There is circumstantial evidence that endotracheal intubation may

lead to prolongation of the Q-T interval. Prolongation of the Q-T interval has been found after the injection of noradrenaline into dogs (Abildskov, 1976). Tomori and Widdicombe (1969) showed that tracheal stimulation causes a sympathoadrenal outflow in cats, and Russel et al (1981) demonstrated increased arterial noradrenaline levels associated with raised blood pressure following laryngoscopy and intubation in humans. Thus laryngoscopy and intubation may cause prolongation of the Q-T interval associated with high serum catecholamine concentrations.
(iv) T wave - T wave changes were seen in 54% of the patients.
Abnormal cardiac rhythm occurred in 3 patients (6%). These were nodal rhythm in one patient, ventricular extrasystoles in a second, and escaped beats during intubation in a third. In all cases these were transient changes and had disappeared by one minute after the intubation.

Forbes and Dally (1970) also reported the development of ventricular extrasystoles during endotracheal intubation (9%), and the flattening and inversion of T waves (5%).

1.1.1.4.1.1.4.2 Neonatal

Apart from the references quoted in the section on neonatal heart rate responses, there were no studies uncovered that dealt specifically with the ECG findings in neonates during laryngoscopy and intubation. In older children (1.5 +/- 0.6

years) Lindgren and Saarnivaaara (1985) showed the following halothane anaesthesia there was very little change in the ECG tracings taken during laryngoscopy and intubation.

1.1.1.4.1.1.5 Cardiac output/index

Laryngoscopy and intubation has been shown to increase cardiac output and cardiac index in anaesthetised (Bernstein et al, 1987; Barash, 1980; Bedford,1981) and awake/sedated patients (Long, 1982).

Bernstein (1987) showed a small increase in cardiac output (0.2 +/- 0.4 litres/minute) following laryngoscopy and endotracheal intubation in his study of 24 healthy patients undergoing elective surgery. All patients in this study were given a thiopentone induction and the duration of laryngoscopy was limited to less than 30 seconds.

Bedford (1981) reported on the responses to laryngoscopy and intubation in 4 groups of patients, each group having been given a different combination of anaesthetic agents prior to the procedure. There were small increases in cardiac index in all of the groups after intubation. The changes were as follows:

Morphine-N2O-O2 group - 3.7 +/- 0.3 litres/minute to 4.7 +/- 0.1 litres/minute (27%)

Innovar-N2O-O2 group - 3.1 +/- 0.2 litres/minute to 3.45 +/- 0.2 litres/minute (11%)

Halothane-N2O-O2 group - 3.1 +/- 0.3 litres/minute to 4.0 +/- 0.3 litres/minute (29%)
Enflurane-N2O-O2 group - 3.0 +/- 0.3 litres/minute to 3.4 +/- 0.8 litres/minute (13%)

Long (1982) compared the cardiovascular responses to laryngoscopy and endotracheal intubation in two groups of patients ie a group labelled "anaesthetised intubation" and a group labelled "awake intubation". The anaesthetised patients were induced with thiopentone, fentanyl, lidocaine, succinyl choline and N2O-O2 prior to intubation. The awake intubation group were given ivi diazepam, fentanyl and lidocaine before intubation.
Cardiac index increased from 3.7 +/- 0.7 litres/minute/m2 to 4.5 +/- 2.1 litres/minute/m2 (22%) at 30 seconds after the intubation in the anaesthetised group. In the awake group there was a similar change from 3.8 +/- 0.8 litres/minute/m2 to 4.4 +/- 1.6 litres/min/m2 (16%). In both groups the cardiac index was still marginally elevated at 3 minutes post intubation.
The results of these studies show small and in most cases insignificant increases in cardiac index. It must be emphasised that specific cardiodepressant drugs were given to these patients in an effort to reduce the sympathetic response to the stimulation. There are no reports detailing the effects of laryngoscopy and intubation in "untreated" or unpremedicated patients, and it is very unlikely that such data will ever become available considering the ethical ramifications.

1.1.1.4.1.1.6 Wedge pressure

The pulmonary capillary wedge pressure (PCWP) has been shown to increase during laryngoscopy and intubation. Bedford (1981) compared the changes in cardiovascular parameters as recorded by thermistor tipped Swan-Ganz catheters, in 4 groups of patients as previously described (see above). The increases over the stimulation period were as follows:

Morphine-N2O-O2 - 8.0 +/- 1.9 mmHg to 10.5 +/- 1.3 mmHg (31%)
Innovar-N2O-O2 - 10.8 +/- 1.1 mmHg to 11.2 +/- 1.4 mmHg (4%)
Halothane-N2O-O2 - 8.2 +/- 1.2 mmHg to 12.6 +/- 1.9 mmHg (54%)
Enflurane-N2o-O2 - 6.7 +/- 2.4 mmHg to 7.4 +/- 2.5 mmHg (10%)

In the study by Long (1982) the PCWP increased from 9 +/- 3 mmHg to 16 +/- 5 mmHg within 30 seconds of intubation in the anaesthetised group, and from 9 +/- 2 mmHg to 13 +/- 2 mmHg over the same time period in the awake group. The PCWP was still significantly elevated above baseline levels at 3 minutes post intubation in both groups.

1.1.1.4.1.1.7 Ejection fraction

Left ventricular ejection fraction changes have been reported following laryngoscopy and endotracheal intubation. Barash and colleagues (1980) used Technecium 99m as a tracer radionuclide and calculated left ventricular ejection fraction changes after laryngoscopy and intubation in 15 patients with ischaemic heart disease. Laryngoscopy and endotracheal intubation (duration of procedure = 13.2 +/- 2 seconds) decreased the ejection fraction from 49% to 32% ($p<0.001$). This was accompanied by 31% and 37% increases in heart rate and blood pressure respectively. In 11 of the 15 patients the ejection fraction returned to pre-intubation levels within 3.1 +/- 0.4 minutes and continued to increase to a maximum ejection fraction of 56% at 5.3 +/- 0.7 minutes following intubation. This data reveals that the magnitude of the left ventricular dysfunction during laryngoscopy and intubation may not be revealed by conventional monitoring methods, and that more sophisticated techniques such as nuclear probes may be needed to fully delineate the cardiovascular disturbances induced by this procedure. Sell and colleagues (1987) also evaluated left ventricular function in a group of patients with ischaemic heart disease undergoing laryngoscopy and intubation. Using radionuclide angiocardiography they showed significant abnormalities in left ventricular wall motion in 3 of 10 patients during the insult. In 2 of these patients the onset of wall motion abnormalities was temporally associated with decreases in

left ventricular wall motion of 15% and 18% respectively. The third patient developed an elevated PCWP (increase of 9 mmHg) when wall motion deteriorated.

These two studies serve to illustrate the potential strain that laryngoscopy and intubation may impose on the myocardium. In a healthy subject such strain may not be of consequence, but in a patient with reduced reserves (caused by acidosis, hypercarbia and hypoxia) there may be disproportionate responses to myocardial stresses of this nature.

1.1.1.4.2 Cerebrovascular effects of L/I

1.1.1.4.2.1 Introduction

The changes in intracranial pressure brought about by laryngoscopy and endotracheal intubation are well recognised and well documented in adults (see below). In most cases however these reports are based on clinical observations, often in patients with underlying cerebral pathology. Clinical research does however demand compromises in protocol and there is less control over variables than in a laboratory setting. This thesis has been designed essentially to have a clinical application, and for this reason certain of the variables have been maintained at unphysiologic levels, despite having been carried out in a

laboratory. The value of the model in this situation is the ability to standardise the variation so that all subjects are exposed to the same change.

Neonates that require intubation and ventilation fall into two major categories, viz. elective intubation and emergency intubation. In both cases there has often been some disturbance of the underlying physiology, whether by the anaesthetic induction and all that goes with it, or by the respiratory failure or severe asphyxia and cerebral oedema seen in many asphyxiated neonates. A frequent scenario in the labour ward is the mildly asphyxiated infant with meconium aspiration. In this particular instance the neonate requires endotracheal intubation not only to establish adequate respiration, but also to enable adequate tracheal toilet to be instituted. In these cases the infant is often adequately oxygenated (due to mask O2 prior to the intubation), mildly hypercarbic as a result of air trapping in atelectatic airways and mildly acidaemic. Another common clinical situation is that of pneumonia where the neonate is well oxygenated in head box oxygen but becomes progressively hypercarbic and acidotic due to decreased lung compliance.

Because of the effect that these underlying conditions may exert, the response studied in this thesis is not a single one, but a composite of both the stimulus and the underlying abnormality. It is this composite that I have attempted to mimic. By superimposing the effects of laryngoscopy and intubation on an underlying disturbed equilibrium, I have sacrificed the ability

to determine the pure effect of the stimulus, but have on the other hand gained the ability to study the changes in a clinically applicable milieu.

Before covering the current literature concerning changes in intracerebral pressure in detail, it is therefore important that some cerebral physiology relevent to the specific disturbances induced, be briefly reviewed.

1.1.1.4.2.2 Cerebrovascular physiology

1.1.1.4.2.2.1 Cerebral circulation

The cerebral circulation is a specialised vascular bed and although several of its regulatory mechanisms (such as its responses to changes in blood gases and pH) are similar to those seen in other areas of the circulatory system, there are some features that are unique.

The blood-brain barrier isolates and protects the the brain, minimising the effects of ionic changes and humoral stimuli on the brain or its vessels.

Vascular resistance within the brain is controlled by the large arteries to a much greater degree than seen in other vascular beds (Heistad and Kontos, 1984). Cerebral vessels are very responsive to changes in arterial pressure and autoregulation is extremely effective. In addition, these blood vessels are highly

sensitive to chemical stimuli as demonstrated by the pronounced vasodilatation produced by hypercapneic acidosis and hypoxia. In contrast the effects of neural stimuli are circumscribed, and often only apparent at times of stress ie the protective effect of the sympathetic nervous system during periods of hypertension.

1.1.1.4.2.2.2 Autoregulation

Autoregulation may be defined as the occurence of vasodilatation as cerebral perfusion pressure decreases, and the occurrence of vasoconstriction as cerebral perfusion pressure increases (Heistad and Kontos, 1984). From a clinician's point of view autoregulation may be defined as the relative constancy in blood flow during alterations in arterial pressure (Edvinsson and MacKenzie, 1977), since it focuses on cerebral function and alterations in function produced by changes in blood flow.
Two main mechanisms, myogenic and metabolic, are believed to account for autoregulation in the brain and other vascular beds (Johnson, 1964). The myogenic mechanism is based on the premise that cerebral vessels are able to alter their degree of contraction or dilatation in response to changes in transmural pressure. The stimulus may be either variation in tension or stretch imposed on the vascular muscle. The metabolic mechanism is based on the premise that vascular muscle activity is dependant on vasodilator metabolites produced in the surrounding tissue. The concentration of such metabolites around blood

vessels can be altered by blood flow. Vasoconstriction would result from an increase in blood pressure because the increased flow would reduce the concentration of vasodilator. Conversely, decreases in blood flow would allow accumulation of vasodilator metabolite, increased dilatation, and a compensatory increase in blood flow. The myogenic mechanism is then seen to be a pressure dependant one, and the metabolic mechanism a flow dependant one. Although there is dissention as to which of these two mechanisms is the more active (Jacobson et al, 1963; Raisis et al, 1979) the current view is that the predominant mechanism responsible for the autoregulation of the cerebral vasculature is metabolic (Raisis et al, 1979; Wagner and Traystman, 1983).

The exact nature of the metabolite or metabolites responsible for the mediation of metabolic autoregulation is not clear, but based on direct measurements of their concentration during blood pressure changes, it is very unlikely that either H+ or K+ mediates metabolic autoregulation (Perez-Hernandez and Anderson, 1976; Matthew et al, 1972; Wahl et al, 1979). Prostaglandins have also been excluded as the responsible metabolites in adults (Pickard, 1973). The currently proposed mediator of autoregulatory adjustments in vessel size may be adenosine, generated in response to tissue hypoxia (Winn et al, 1980; Wahl et al, 1976).

Carbon dioxide has been shown to affect the autoregulation of brain blood flow (Rapela and Green, 1964; Haggendal, 1965), and this holds true in neonatal piglets when the CO2 tension is

maintained at hypercapneic levels (69 +/- 3 mmHg). Stonestreet et al (1985) reported a tendency to a pressure passive circulation during hypercarbia, with the most pronounced effect being in the brain stem. The greatest degree of vasodilatation was also shown to occur in the brainstem.

1.1.1.4.2.2.3 Neural regulation

Cerebral blood vessels are well supplied with nerves, but the role of neural mechanisms in the regulation of cerebral blood flow has been controversial (Heistad and Marcus, 1978; Purves, 1972). Cerebral vessels receive sympathetic innervation mainly from the superior cervical ganglion (Nielsen and Owman, 1967). The nerves supplying the cerebral vessels originate primarily in the ipsilateral ganglia, but arteries in basal and medial areas receive bilateral innervation. The sympathetic system innervates large and small arteries (pial and parenchymal) as well as the corresponding veins (Nielsen and Owman, 1967). The heterogeneity of the innervation has been studied and it appears that the arteries in the internal carotid system are more extensively supplied than those of the vertebral system (Edvinsson, 1975). Cholinergic nerves have been shown to supply surface arteries of the brain, but cholinergic innervation of intraparenchymal cerebral vessels has not been established (Edvinsson, 1975). The origin of the cholinergic innervation is uncertain, but the greater superficial petrosal nerve, a branch of the seventh

cranial nerve, has been shown to provide part of the cholinergic innervation of the cerebral vessels (Vasquez and Purves, 1979). The close approximation of adrenergic and non-adrenergic, presumably cholinergic, nerve terminals in the cerebral arteries is an indication that there is an interaction between cholinergic and sympathetic nerves in the cerebral vessels (Owman, Edvinsson and Nielsen, 1974).

Recent work has shown vasoactive intestinal peptide in nerves on cerebral vessels (Larsson et al, 1976), but anatomical pathways have yet to be established.

Cerebral blood vessels have some major differences in terms of the adrenergic receptors when compared to other vessels. The alpha-adrenergic receptors are relatively insensitive to noradrenaline (Toda and Fujita, 1973) and the ED50 for noradrenaline is at least 100 times greater in the basilar artery than in the saphenous artery. In contrast the responses to serotonin are similar for the two vessels (Bevan, Duckles and Lee, 1975).

The available evidence indicates that alpha-receptors in cerebral vessels are less discriminating and less sensitive to agonists than alpha-receptors of other vessels (Heistad and Kontos, 1984). There is evidence to show that differences in extracellular and intracellular calcium may contribute to the differences in responsiveness of cerebral and other systemic

arteries. Basilar and saphenous arteries have been demonstrated to to have significant differences in the relative dependence on intracellular and extracellular calcium sources (Towart, 1981). Another possible explanation for the differences in sensitivity of cerebral and extracranial arteries to adrenergic stimuli may relate to their different embryonic origin. Intracranial and extracranial arteries arise from different primordial cells, and the site of fusion of the arteries corresponds to the change in responsiveness to noradrenaline (Bevan, Duckles and Lee, 1975).

Wagerle and Delivoria-Papadopoulos (1987) have demonstrated alpha-1 and alpha-2 adrenoreceptor mediated vasoconstriction in the cerebral circulation of newborn piglets, but not in adult pigs, supporting the concept that there are developmental differences in cerebrovascular neuroeffector mechanisms in this species. Because of discrepancies in the vasoconstrictor response when pial vessels are examined separately from the total cerebrovascular bed (Busija and Leffler, 1987) there is the possibility of segmental differences in the population of alpha-adrenoceptor subtypes that mediate vasoconstriction following sympathetic stimulation (Wagerle and Delivoria-Papadopoulos, 1987). These authors suggest that large cerebral arteries may be predominantly alpha-1 receptor mediated, while smaller arterioles of the pial circulation may be predominantly alpha-2 mediated. This hypothesis has important implications for the control of cerebral blood flow (CBF) since large conducting arteries including the internal carotid artery, Circle of Willis,

and large pial arteries contribute significantly to the regulation of cerebral blood flow (CBF) (Heistad and Kontos, 1984), and adrenergic neuroeffector mechanisms that alter large artery resistance will be reflected in changes in CBF.

Heistad and Kontos (1984) make the point that in studies of the effects of catecholamines on cerebral vessels it is essential that direct effects on the vessels are separated from indirect effects mediated by changes in arterial pressure. The blood-brain barrier limits access of catecholamines to cerebral vascular smooth muscle during intravascular administration of amines (MacKenzie et al, 1976), and intracarotid infusion of noradrenaline has little effect on CBF. After disruption of the blood-brain barrier, however, intracarotid infusion of noradrenaline produces an increase in flow (MacKenzie et al, 1976), which is apparently secondary to an increase in cerebral metabolism.

The physiological role of sympathetic nerves arising from the superior cervical sympathetic ganglion is not well defined (Heistad and Marcus, 1978; Purves, 1978), but there is now good evidence to support the view that in specific circumstances they are of importance. Such conditions include acute hypertension and hypercapnia where sympathetic nerves may regulate intravascular pressure in cerebral microvessels, protecting against sudden increases in blood pressure (Bill and Linder, 1976). Increases in blood flow during severe hypertension and the protective effects of sympathetic stimulation are more pronounced in grey matter

than in white matter (Heistad and Marcus, 1979). Mechanisms contributing to augmented cerebrovascular responses to sympathetic stimulation during acute hypertension are not clear. Sudden increases in arterial pressure, within the physiological pressure range, produce transient increases in CBF, and sympathetic stimulation has been shown to attenuate this transient hyperaemia (in cats) (Busija, Heistad and Marcus, 1980).

In addition to CBF effects the sympathetic nerves also have other effects on the cerebral vessels. These include modulation of the rate of formation of the cerebro-spinal fluid (Lindvall, 1978), and protection of the blood brain barrier during acute hypertension (Bill and Linder, 1976) by attenuating the increase in permeability to albumin, particularly in the cerebral grey matter (Heistad and Marcus, 1979). Sympathetic pathways to cerebral vessels have also been shown to be activated during systemic hypercapnia, and dilatation of large vessels during hypercapnia is attenuated by these nerves (Wei et al, 1980).

There are several studies suggesting an important role for the sympathetic system in the newborn period (Busija and Leffler, 1987; Busija, Leffler and Wagerle, 1985; Goplerud, Wagerle and Delivoria-Papadopoulos, 1987; Kurth, Wagerle and Delivoria-Papadopoulos, 1986; Wagerle, Kumar and Delivoria-Papadopoulos, 1986). Kurth et al (1986) suggested that reflex action of sympathetic nerves during seizures in neonatal lambs decreased the CBF, and Goplerud et al (1987) showed the same effect during

apnoea in newborn piglets. Wagerle et al (1986) showed that sympathetic stimulation decreases blood flow to the grey and white matter, hippocampus, and choroid plexus, and that this reduction in blood flow is augmented during hypercapnia.

The role of cholinergic nerves in the regulation of CBF is unclear, but it is likely that the steady state vasodilation accompanying hypoxia and hypercapnia is the result of local metabolic mechanisms rather than neural ones (Heistad and Kontos, 1984). Nerves may modulate the time course of the cerebral vasodilatation, but it is still unclear from morphological studies whether non-adrenergic innervation is sufficiently dense or widespread enough to have any major effect on cerebral blood flow (Heistad and Kontos, 1984).

1.1.1.4.2.2.4 Chemical Regulation

1.1.1.4.2.2.4.1 CO2 and pH

The chemical regulation of CBF is mainly controlled by oxygen, carbon dioxide and blood pH.
Carbon dioxide and hydrogen ions (H+) have a pronounced relaxant effect on the cerebral vascular smooth muscle. Arterial hypercapnia dilates pial arterioles, and hypocapnia constricts the vessels (Wolff and Lennox, 1930; Wei et al, 1980). This effect has been shown to be dependant on the vessel size, with

increases in PCO2 producing a greater percentile increase in the radius of small vessels than large vessels (Wei et al, 1980). The effect of CO2 has been shown to be mediated by a change in extracellular fluid pH, and not from any direct vasoactive effect of its own (Heistad and Kontos, 1984).

Low pH relaxes cerebral vascular muscle and high pH contracts the muscle (Edvinsson and Sercombe, 1976), these changes being mediated by H+ which has been shown to have a direct relaxant effect on the muscle. Hydrogen ion concentration in the immediate vicinity of the vascular muscle is dependant on the HCO3- concentration and on the PCO2 of the extracellular fluid in the area. The extracellular fluid PCO2 is in turn dependant on the arterial and CSF CO2 tensions. The blood brain barrier is impermeable to H+ and HCO3-, but is freely permeable to molecular CO2. When the PaCO2 is increased, molecular CO2 diffuses across the blood brain barrier, increases the local PCO2 of the vascular muscle, and reduces the extracellular fluid pH in that area, producing vasodilatation by relaxation of the vascular muscle. The reverse occurs when the PaCO2 is lowered. This model was first suggested by Gotoh, Tazaki and Meyer (1961).

Hypercapnia increases CBF and blood volume and decreases cerebral vascular resistance (Heistad and Kontos, 1984). The magnitude of the responses to the changes in PCO2 differ in different regions of the brain. Increases in blood flow are more marked in the cerebral grey matter than in the white matter, and percentile increases in flow are pronounced in the medulla (Heistad et al,

1976). Decreases in the rate of oxygen consumption reduce the hyperaemic responses to hypercapnia because of the decrease in the rate of production of CO2.

The relationship between total CBF and PaCO2 is curvilinear (Reivich, 1964) with the slope being steep in the midrange and flattened at both the high and low levels of CO2. Maximum increases in flow rate have been variously reported as occurring at PaCO2 levels >60mmHg (Marcus, Bischof and Heistad, 1979), 80mmHg (Harper and Glass, 1965) and 121mmHg (Reivich, 1964). During hypercapnia CBF becomes more dependant on arterial blood pressure, and it would probably be more appropriate to consider maximal cerebral vasodilator responses in terms of vascular resistance rather than blood flow under these circumstances.

Since the major mechanism of action of CO2 on the cerebral circulation is mediated by changes in CSF pH, changes in blood pH during a constant PaCO2 have negligible effects on cerebral blood flow and cerebral vascular resistance (Harper and Bell, 1963; Wagerle et al, 1988).

In terms of other mechanisms mediating the vasodilator or vasoconstrictor responses of cerebral vascular muscle to CO2, there is no conclusive evidence that either direct neurogenic influences via the vasomotor nerves, or that other substances such as prostaglandins have any part to play in adults (Heistad and Kontos, 1984). Wagerle and Mishra (1987) presented data

supporting a role for arachidonic acid metabolism and production of vasoactive prostaglandins mediating the cerebrovascular response to hypercapnia in the newborn piglet. They also suggested a mechanism by which extracellular H+ may influence cell membrane function and activate phospholipase to release arachidonic acid.

With specific reference to the neonatal piglet model, Hansen et al (1984) studied the effects of variations in PaCO2 on the brain blood flow and cardiac output in the newborn pig. They found that brain blood flow was maintained during hypocarbia until extremely low PaCO2 values (<15mmHg) were achievd, at which time total brain and cerebral blood flow decreased significantly (40%) from baseline levels. Blood flow to the thalamus, cerebellum and brain stem was unchanged from baseline during hypocarbia, suggesting that the newborn brain is relatively insensitive to moderate degrees of hypocarbia. Even at extreme levels of hypocarbia (<15mmHg) the cardiac output was maintained, despite an increase in heart rate and a decrease in arterial pressure.
Hypercarbia with normoxaemia was associated with significant increases in total brain blood flow, as reported in studies with adults. A larger proportion of the increased brain blood flow was distributed to the brain stem, cerebellum and thalamus, than to the cerebrum. The percentage of the cardiac output received by the brain was also increased, despite the fact that the total cardiac output remained unchanged. This study showed that the neonatal piglet cerebral vasculature is sensitive to hypercarbia

and that regional differences in sensitivity may account for the greater increases in blood flow to the basal portions of the brain, than to the cerebrum (Hansen et al, 1984).

In another study, Brubakk et al (1987) demonstrated a difference in the response to prolonged hypercarbia in the newborn piglet when compared to the adult. They state that in the adult, hypercarbia-induced cerebral hyperaemia may recede to normal, possibly as a result of the modulating effect of normalization of brain tissue pH. They found that in newborn piglets total brain blood flow increased significantly in response to hypercarbia (66 +/- 5 mmHg), decreased slightly at 2 hours, and was significantly less than the 0.5 hour value at 4 hours of hypercarbia. Although the decrease in brain blood flow occured at 4 hours there was no trend to normalization and the flow rate was still significantly elevated.

Not only does hypercarbia increase the total brain blood flow in neonatal piglets, but there are increases in mean arterial pressure (65mmHg - 85 mmHg over 30 minutes) that also follow hypercarbia (Brubakk et al, 1987). This increase in arterial pressure has been shown to be related to increases in catecholamine release (adrenaline) believed to be mediated through concurrent metabolic acidosis (Brubakk et al, 1983).

Wagerle et al (1986) studied the effects of electrical stimulation of the sympathetic trunk in normocarbic and hypercarbic neonatal piglets. They demonstrated that during normocarbia activation of the sympathetic nerves has minimal effect on cerebral blood flow, but profoundly decreases choroid

plexus blood flow. During hypercapnia, however, activation of the sympathetic nerves may severely attenuate the vasodilatory capacity of the cerebrovasculature and significantly reduce the increased cerebral blood flow that accompanies hypercarbia, without any effect on the blood pressure. This demonstrates that the vasoconstriction resultant from sympathetic stimulation can modulate the vasodilatory effects of hypercarbia. This is an important point, and will be referred to in the discussion of the possible mechanisms responsible for the changes seen in this series of experiments following laryngoscopy and intubation.

1.1.1.4.2.2.4.2 Oxygen

Arterial hypoxaemia induced by the inhalation of gas mixtures containing low concentrations of O2 causes pial arteriolar dilatation (Wolff and Lennox, 1930). At low O2 tensions, in the range of 20 - 30 mmHg, vasodilatation is pronounced and comparable with the maximal response seen during hypercapnia (Wolff and Lennox, 1930). Oxygen has been demonstrated to alter the activity of the smooth muscle of the pial arteries by local mechanisms, and these local mechanisms have been shown to be the most important regulators of cerebrovascular tone during hypoxia (Kontos et al, 1978). The exact mechanism by which tissue hypoxia leads to pial arteriolar vasodilatation is unclear, but adenosine (released from neural parenchyma), H+ and K+ are all believed to have some role (Winn et al, 1981). In neonatal piglets there is

evidence to suggest that metabolites of the cyclo-oxygenase pathway may play a role in the regulation of cerebrovascular tone during hypoxia as well (Wagerle, Mishra and Delivoria-Papadopoulos, 1985).

Increases in PaO2 above normal induce modest pial arteriolar constriction (Kontos et al, 1978; Kety and Schmidt, 1948; Lambertsen et al, 1953; Wolff and Lennox, 1930). Jacobsen et al (1964) showed in studies on adult dogs, in whom alveolar PCO2 was kept constant, that a 15% reduction in CBF occurred during the inhalation of 100% oxygen.

Hyperoxia (PaO2 = 349mmHg) in newborn puppies was shown to reduce cerebral blood flow by 20 - 30 % in 2 day old animals, with significant reductions in 23 of the 35 regions reviewed (Kennedy, Grave and Jehle, 1971). In 3 week old puppies hyperoxia resulted in less marked reduction in CBF than in the 2 day old dogs, with significant decreases seen in only 8 of the 35 areas studied. In addition age appeared to decrease the sensitivity of the cerebral circulation to oxygen, especially in the white matter; hyperoxia reduced blood flow in the white matter by an average of 11% in the 3 week old animals, compared with an average reduction of 24% in the 2 day old puppies (Kennedy, Grave and Jehle, 1971). Conclusions from this data should be made bearing in mind the relative immaturity of the dog at birth, and it may be that the CBF values seen at 3 weeks in the dog are more appropriate for extrapolation to neonates in other species.

In a study on adult rat local cerebral blood flow responses to hyperoxia and hypoxia, Shinozuka and Nemoto (1981) showed that 10 - 20% increase occurred in the local cerebral blood flow (CBF) when the PaO2 was decreased from 250 mmHg (control) to 130 mmHg. At PaO2 levels below 130 mmHg, there was a large increase in local CBF such that at a PaO2 of 80 mmHg, local CBF was 180% of the control, and at 40 mmHg local CBF was 240% of control. In the same study the brain tissue H+ was shown not to change over the PaO2 range 100 - 200 mmHg.

The differing effects of high PaO2 tension in neonates and adults or older newborns, suggests that local factors in the different brain areas may be more important in explaining the degree of reactivity, than any specific property of the vasculature itself. Oxygen is well known to be one of the factors capable of influencing vessel tone by alterations in its tissue levels (Lassen, 1959). During early development all cerebral structures have a sharply rising metabolic rate, but the rate of change and the time at which thay reach maximal levels are varied for different regions (Kennedy et al, 1971). This changing pattern can be expected to result in corresponding variations in local tension in respiratory gases and pH. The finding of blood flow values that are uniformly lower in oxygen-exposed newborns than those in air-exposed newborns, could be accounted for by a flooding with oxygen of tissue characterised at this age by a relatively low oxygen requirement. The resulting increase in PO2 would cause marked vasoconstriction. At an older age, when a rise in oxygen consumption has taken place in many regions, a similar

flooding of the tissues with oxygen may result in greater variation in regional PO2, with areas of high oxygen consumption acting as local sinks. In such regions the oxygen tension may not increase to the same degree, and vasoconstriction might be less than in areas of lower consumption. It has been shown that in white matter there is a reduced effect of hyperoxia at 3 weeks following birth, a time when metabolic requirements are maximal and myelin formation at a peak (Kennedy et al, 1970). This might explain the different effects of hyperoxia seen at different ages, and in different species with varied levels of development, at birth.

1.1.1.4.2.3 Adult

Changes in cerebrospinal fluid pressure have been demonstrated in adults during laryngoscopy and tracheal intubation (Stephen et al, 1954); Shapiro et al, 1972). The rise in intracranial tension may be as much as 100mmHg (Greenbaum et al, 1975) although most other workers have found increases of a lesser degree (Shapiro et al, 1972; McLeskey et al, 1974; Misfeldt et al, 1974; Burney and Winn, 1975; Moss et al, 1978). This increase in intra-cranial tension has been related to a number of factors which could be associated with laryngoscopy and endotracheal intubation including raised arterial blood pressure (Stephen et al, 1954;

Moss et al, 1978), elevated central venous pressure (Moss et al, 1978; Hunter, 1952), and anaesthetic drugs (Greenbaum et al, 1975; Misfeldt et al, 1974).

The rise in arterial pressure seen following laryngoscopy and intubation in adults does not significantly alter cerebral blood flow if the arterial pressure changes are within the range 60 - 150 mmHg (McCleskey et al, 1974). However it has been suggested that the acute hypertension seen at laryngoscopy and intubation can induce rapid cerebral swelling in patients with underlying acute cerebrovascular problems (Alexander and Lassen, 1970). Patients with pre-existing intracranial hypertension (such as may be expected with asphyxia or prologed acidosis) may experience undesirable surges in brain blood flow when an increase in pressure occurs, with the possibility of transcompartmental herniation of brain tissue (Shapiro et al, 1972).

1.1.1.4.2.4 Neonatal

Transient changes in intracranial pressure in healthy neonates are probably of little significance. Increases in intracranial tension in an asphyxiated neonate with any element of cerebral oedema or raised intracranial pressure, however, are certainly of importance, and the potential for exacerbated cerebral injury is heightened. As mentioned above the additional pressure rise induced during laryngoscopy and intubation may lead to worsened cerebral oedema (Alexander and Lassen, 1970). Once the mean

arterial pressure has returned to normal, however, the increased intracranial pressure resulting from the oedema may severely decrease the cerebral perfusion pressure. Lou et al (1979), have shown that in asphyxiated neonates with reduced cerebral blood flow (low cerebral perfusion pressure) the neurologic outcome is uniformly poor.

The present study has been aimed at the asphyxiated or distressed neonate where some degree of cerebral injury may exist at the time of resuscitation. It has been shown (Brubakk et al, 1987) that certain conditions (hypercarbia) may lead to a pressure-passive cerebral vasculature, which would make any sudden increase in cerebral blood flow (and consequently intracranial pressure) of great significance in terms of extension of pre-existing damage.

In preterm neonates increased intracranial pressure (ICP) during laryngoscopy and intubation has been associated with coughing and forced expiratory efforts. Friesen et al (1987) demonstrated that anterior fontanel pressure increases significantly during awake intubation in preterm neonates. Anterior fontanel pressure (AFP) has been shown to be a reliable indicator of intracranial pressure (Hill and Volpe, 1981), and the authors thus concluded that awake endotracheal intubation causes significant increases in intracranial pressure. The observations of Perlman et al (1985) support the theory that venous stasis accompanying frequent spasms of coughing and sustained forced expiration

increases cerebral blood volume and intracranial pressure. They noted wide fluctuations in cerebral blood flow velocity in preterm neonates whose spontaneous ventilatory efforts were out of synchrony with their ventilators.

Such fluctuations in ICP following awake intubation were also demonstrated by Raju et al (1982) who showed that ICP increased by between 5 and 20 mmHg during crying in healthy term newborn babies. In preterm babies the magnitude of the ICP increase was greater possibly because of differences in the vigour of the crying, and poorer maturation of the autonomic nervous system with lack of smooth muscle in the arterioles. Raju et al (1982) concluded that such changes may be of very little significance in healthy babies, but that repeated crying and struggling in response to some painful or unpleasant stimulus in sick neonates might influence their cerebral oxygenation.

Increases in AFP are, however, not confined to neonates subjected to laryngoscopy and intubation whilst awake, and there is work to show that there is a significant increase in AFP following the stimulus in anaesthetised neonates. In a study of the intracranial pressure effects of laryngoscopy and intubation in both awake and anaesthetised neonates, Stow et al (1988) demonstrated significant increases in AFP in both groups. The authors compared the AFP changes of awake intubation with those following laryngoscopy and intubation after a thiopentone/ atropine/suxamethonium induction of anaesthesia. The increase in AFP in the anaesthetised neonates (8.7 +/- 0.8 mmHg to 15.8 +/- 18 mmHg) was significantly less than in the awake group (9.6 +/-

0.5 mmHg to 33.5 +/- 3.6 mmHg). There was still, however, a significant increase in AFP in the anaesthetised group when the post laryngoscopy level was compared to the baseline. Raju and colleagues (1980) also found a significantly greater increase in AFP in infants undergoing awake intubation compared with those paralysed before intubation. In their study on nasotracheal intubation in neonates, Kelly and Finer (1984) reported an average increase of 19.8 mmHg in the AFP following intubation in their control group.

1.1.1.4.3 Endotracheal suctioning

Endotracheal suctioning in adults has been shown to increase intra cranial pressure, and in their review of the subject Rudy et al (1986) concluded that the available evidence demonstrates such increases in intracranial pressure. The elevation appears to be transient, with a rapid return to baseline values in most patients. In the majority of cases the mean arterial pressure is also elevated with suctioning, but the exact relationship is unclear. The elevation in intracranial pressure has been seen to be most pronounced in patients who already have intracranial hypertension: the very patients least able to tolerate such elevations.

Endotracheal suctioning also produces increases in cerebral blood flow velocity (Perlman and Volpe, 1983) and intracranial pressure (Fisher et al, 1982) in neonates and infants. Friesen et

al (1987) likened the stimulation of endotracheal suctioning to that of endotracheal intubation, basing his study of the effects of tracheal intubation on anterior fontanel pressure. Fisher et al (1982) concluded that the increase in intracranial pressure seen in children during endotracheal suctioning was the result of tracheal stimulation, rather than increases in carbon dioxide tension. In their study the levels of oxygen and CO2 were controlled and at no stage did the CO2 rise above normal.

The interpretation of the cerebrovascular effects of endotracheal suctioning in most studies is difficult, because in almost all studies there are significant variations in both oxygen and carbon dioxide tension, both of which have considerable effects on the cerebral vasculature and arterial blood pressure. Durand et al (1989) reported on the cardiopulmonary and intracranial pressure changes related to endotracheal suctioning in preterm neonates. In their study however the suctioning was carried out through a suction adaptor on the endotracheal tube and the patients were not disconnected from their ventilators. This practice minimized the hypoxaemia and hypercapnia usually associated with endotracheal suctioning. Their findings indicate that endotracheal suctioning significantly increases mean arterial blood pressure (36 mmHg - 48 mmHg), intracranial pressure (6.2 - 13.6 mmHg), and cerebral perfusion pressure (32 - 40 mmHg), and that these changes are independent of oxygenation and ventilation.

Although it is obvious that tracheal stimulation is involved in the response to suctioning, it is easier to deduce an aetiology from studies that limit the variables affected by the stimulus. For this reason a detailed review of the literature pertaining to endotracheal suctioning has been avoided, and the literature survey confined to reports related to laryngoscopy and intubation.

1.1.1.4.4 Respiratory effects of L/I

1.1.1.4.4.1 Reflex Responses

1.1.1.4.4.1.1 Introduction

The larynx, because of its strategic location at the crossroad between the alimentary and respiratory tracts, can be viewed as subserving a fundamental protective function for the lower respiratory tract. Protective reflexes originating from the larynx include cough, apnoea, the expiration reflex, swallowing, bronchoconstriction, and mucus secretion. The role of these reflexes is primarily to prevent inhalation of damaging agents into the laryngeal lumen, or, when inhalation has already occurred, to expel them. In some instances however, the real purpose of the reflex is unclear, as in the case of bronchoconstriction and some of the cardiovascular responses that

are obviously damaging. The density of sensory endings described in various laryngeal structures is consistent with the extent of the reflex responses. The identification of a particular type of receptor in relation to a specific response remains conjectural. Numerous afferent terminals have been isolated in the larynx. There are free endings, both myelinated and unmyelinated, distributed amongst the epithelial cells, as well as more organised structures like corpuscles and taste buds. These receptors are mostly concentrated in the laryngeal surface of the epiglottis, the glottis, and the subglottic area just below the vocal cords (Widdicombe, 1981).

All of the protective reflexes originating from the larynx are essentially eliminated by section of the superior laryngeal nerve (Korpas and Tomori, 1979).

1.1.1.4.4.1.2 Cough

Although the exact nature of the receptors mediating cough remains uncertain, the rapidly adapting receptors activated by known tussigenic stimuli are generally considered as the probable source (Korpas and Tomori, 1979, Widdicombe, 1977). These endings usually have an intraepithelial location, but it is interesting to note that the vocal folds (a very sensitive area for initiating cough) are, at least in the cat, devoid of nerve terminals within the superficial layer (Jeffery, 1978). Cough elicited from the larynx appears to be similar in its fundamental

characteristics to that evoked from the trachea, but tussigenic stimuli are represented by several different forms of irritation, and can to some extent generate different patterns of coughing. Laryngeal coughing is described as having a stronger expiratory activation than that elicited from the tracheobronchial tree (Tomori and Widdicombe, 1969). Mechanical stimulation of the vocal folds can elicit a purely expiratory activation accompanied by laryngeal constriction - the so-called expiration reflex, much more resistant to general anaesthetics than the cough reflex itself (Korpas, 1979). Weak mechanical stimulation usually induces only a laryngeal closure in expiration, and does not alter the breathing pattern. It is only with stronger stimuli that respiration is inhibited and the other reflex effects are seen (bronchoconstriction, hypertension, and bradycardia) (Widdicombe, 1977). The graded nature of the response could reflect a progressive involvement of different types of receptors, or an increased recruitment and/or faster discharge rate of the same type of endings.

An interesting aspect of airway reflex physiology is the importance that the composition of the airway surface fluid has on various functions, viz motility of the cilia, mucus transport, receptor activity, and hence reflex responses (Higganbottam, 1984). Inhalation of aerosolised water or low-chloride water has been found to induce cough (Eschenbacker et al, 1984, Higganbottam, 1984). For this reason cough has been thought to be important for the preservation of the ionic composition of the airway surface liquid (Higganbottam, 1984). This effect

(cough) is interesting in its similarity to the apneic response evoked by the application of water to the larynx of a neonate, a response supposedly mediated by specific laryngeal receptors (Boggs and Bartlett, 1982). The relevence of these two responses will become more apparent later, when the effects of aerosolised saline and lignocaine, sprayed onto the vocal cords of the experimental piglets prior to intubation, are discussed.

1.1.1.4.4.1.3 Bronchoconstriction

Mechanical and chemical irritation of the laryngeal mucosa may elicit bronchoconstriction leading to an increase in total airway resistance. This resistance develops after a longer delay and persists for a longer time than does cough or apnea (Tomori and Widdicombe, 1969). The laryngeal mucosa is a powerful reflexogenic site for bronchoconstriction, second only to the tracheobronchial airways (Tomori and Widdicombe, 1969). Dohi and Gold (1979) showed that irritation of the brocho-carinal area and insertion of an endotracheal tube, produced a significant increase in pulmonary resistance and a small decrease in pulmonary compliance. These effects were not influenced by other factors known to affect pulmonary mechanics, such as hypoxia (Nadel and Widdicombe, 1962) and changes in PaCO2 (Newhouse et al, 1964). There are cough receptors located in the tracheal bifurcation and in the area of bronchial branching (Widdicombe

and Sterling, 1970), and the increased pulmonary resistance is believed to be related to stimulation of these receptors.

1.1.1.4.4.1.4 Mucus Production

Mucus production is augmented reflexly by laryngeal irritation. This is due to an increase in both sympathetic and parasympathetic activity (vagal 'Type III' fibres), and is believed to involve both submucosal glands and goblet cells (Gallagher et al, 1975). Mucus production is an important defense mechanism and can, in fact, induce coughing (Widdicombe, 1977).

1.1.1.4.4.1.5 Swallowing

Swallowing is both an alimentary and a protective reflex that can be elicited from different parts of the upper airways. The major site of initiation of the swallow is the laryngeal region (epiglottis included), and the most effective stimulus for initiating a swallow (in decerebrate animals) is stimulation of the laryngeal afferents by water (Storey, 1976).

1.1.1.4.4.1.6 Developmental aspects

The defense responses mediated through the superior laryngeal nerve have also been shown to be age-dependent. Water or solutions of low-chloride concentration instilled into the larynx of certain neonatal animals causes sustained apnea and swallowing (Boggs and Bartlett, 1982). This response becomes less pronounced with age and in adults there is only transient respiratory inhibition and/or coughing (Boggs and Bartlett, 1982; Sullivan, et al, 1978; Johnson et al, 1975). The receptors mediating the reflex apnea appear to be the superficial intraepithelial free endings (Harding, 1978). Boggs and Bartlett (1982) showed that the receptors are equally active in neonates and adults suggesting that the difference in reflex response lies in the central integrative process. Of note, and of particular clinical relevence, is the fact that in neonates with viral upper airway infections there is a greater incidence of apnea following laryngeal irritation (Bruhn et al, 1977).

Early in development the afferent impulses originating from receptors located in the mucosa and deeper structures of the larynx are particularly important for the act of swallowing. The superficial receptors appear to mediate this response (Sumi, 1975). When the feedback from mucosal receptors is eliminated in neonates by topical anaesthesia, there is a great reduction in the motor discharges associated with swallowing. No such change occurs in the adult (Sumi, 1975).

A greater developmental change has been documented for tracheobronchial cough than for laryngeal cough (Korpas and Tomori, 1979). Tracheobronchial cough is weak in newborn animals and in preterm infants, with the adult response only being achieved several days to weeks later. Laryngeal cough is only slightly weaker in neonates than in adults (Korpas and Tomori, 1979). This differential development is important in the light of the data to be presented. Since very little work has been published regarding the reflex activity following laryngeal irritation and endotracheal intubation, there has been extrapolation from the observed effects of tracheal and bronchial suctioning. In the light of the above presented findings the reflex activity following laryngeal stimulation (laryngoscopy) in neonates should be more pronounced than that seen with tracheobronchial irritation (suctioning).

1.1.1.4.5 Hormone Response to Intubation

1.1.1.4.5.1 Catecholamines

Russel et al (1981) measured plasma catecholamine concentrations in arterial blood following laryngoscopy and intubation. They found a significant increase in noradrenaline concentration (34%) at one minute after intubation, but no significant change in adrenaline or dopamine levels. The authors concluded that laryngoscopy and intubation is associated with a significant

increase in sympathetic nerve activity. This theory is supported by the work of Tomori and Widdicombe (1969) who showed significant increases in sympathetic nerve activity during mechanical stimulation of the laryngo-bronchial tree in cats.

Derbyshire et al (1983) also demonstrated significant increases in plasma noradrenaline concentration (74%) in association with a significant pressor response following intubation. Patients given suxamethonium for muscle relaxation showed a greater pressor response following intubation when compared with patients given pancuronium. In addition, the suxamethonium group also showed significant increases in plasma adrenaline concentration. These results suggest that tracheal intubation is accompanied not only by increased sympathetic activity, but also by increased sympathoadrenal activity.

Shribman et al (1987) demonstrated that laryngoscopy alone generates the same pressor response and sympathoadrenal responses (as measured by circulating catecholamine concentrations) as laryngoscopy followed by intubation. These workers showed similar and significant increases in both plasma noradrenaline and adrenaline concentrations at 1 minute after laryngoscopy with or without intubation. Noradrenaline concentrations remained significantly increased at 3 minutes after laryngoscopy in both groups. This study confirms the findings of Russel et al (1981) and Derbyshire et al (1983) showing that changes in plasma noradrenaline concentration are linked with changes in mean

arterial pressure. The association between changes in plasma adrenaline concentration and heart rate are less well demonstrated; in Shribman's study there was a significant increase in plasma adrenaline concentration in both the laryngoscopy and the laryngoscopy/intubation groups, but in only the laryngoscopy and intubation group was an increase in heart rate seen. In neither the Russel et al (1981) study nor the Derbyshire et al (1983) study was there a significant correlation between plasma adrenaline concentration and heart rate or pulse pressure.

1.1.1.4.6 Methods of limiting responses

1.1.1.4.6.1 Introduction

The presented data clearly illustrate that the untoward cardiovascular and cerebrovascular effects of laryngoscopy and intubation are well recognized. Measures to attenuate and blunt these responses are many and varied, but as yet no particular protocol has been completely successful or applicable. Many of the drugs that have been used including heavy premedication, high dose narcotics, deep inhalational anaesthesia, and potent vasoactive drugs (Stoelting, 1977; Stoelting and Peterson, 1976; Venus et al, 1984) commonly prolong recovery time and can themselves lead to cardiovascular complications.

Very little has been written about decreasing the potentially adverse effects of laryngoscopy and intubation in neonates, and until recently awake intubation was used by many as a standard method of elective intubation (Berry and Gregory, 1987).
The following section will review the current literature on the methods in use to limit these responses, but will concentrate on reports concerning the use of lignocaine.

1.1.1.4.6.2 Lignocaine

The topical application of lignocaine to the oropharynx has been shown to ameliorate the pressor response to laryngoscopy when given 5 - 10 minutes before the procedure. In addition lignocaine applied intratracheally immediately before intubation, has been shown to reduce the additional pressor componant associated with the insertion of the tube (Denlinger, Ellison, and Ominsky, 1974; Stoelting, 1977; Hamil et al, 1981). In many of the studies reported there have been unconventional methods of induction and in most cases there is no mention made of catecholamine respones to the procedures. In two recent studies the efficacy of topically applied lignocaine has been questioned (Derbyshire, Smith and Achola, 1987; Laurito et al, 1988). Derbyshire, Smith and Achola (1987) studied 30 healthy women undergoing elective gynaecological surgery. They divided the patients randomly into 3 groups: group 1 received 4% lignocaine (160 mg) using a Forrester spray; group 2 received 4% lignocaine (160mg) by a

commercially available spray device; and group 3 received an equal volume of saline administered by a Forrester spray. In all three groups there were similar and statistically significant elevations in mean arterial pressure, plasma adrenaline, and noradrenaline concentrations following the stimulation. There were no statistically significant differences between the groups at any stage. The authors concluded that topical anaesthesia of the mucosa of the upper airways, as performed conventially, is ineffective as a means of diminishing the pressor and catecholamine responses to laryngoscopy and intubation in adults. Laurito et al (1988) carried out a well designed randomized, double-blind study on 40 unpremedicated, (American Society of Anesthesiologists Classification) ASA I - II adult surgical outpatients. They assessed the effects of aerosolized lignocaine, and intravenous lignocaine, alone and in combination, on the circulatory responses to laryngoscopy and intubation. Lignocaine (4mg/kg) or saline was given by nebuliser 15 minutes before induction of anaesthesia. The patients then had a standardised induction of anaesthesia using curare (3mg) and facemask oxygen, followed at 2 minutes by thiopentone (5mg/kg) and succinylcholine (1.5mg/kg). Lignocaine (2mg/kg) or saline was given as an iv bolus at 4 minutes. Laryngoscopy was started at 5 minutes and continued for 45 seconds before intubation. Heart rate and systolic, diastolic and mean arterial pressures were recorded using a non-invasive method, at one-minute intervals from 0 to 11 minutes. There were four treatment groups: group 1 received aerosolized and iv saline; group 2 received aerosolized saline

and iv lignocaine; group 3 received aerosolized lignocaine and iv saline; and group 4 received aerosolized and iv lignocaine). All groups were similar in all measured parameters before the start of the study. Within each group all parameters increased significantly after intubation. The maximum values attained after intubation did not differ significantly among the 4 treatment groups for any of the measured variables. These findings confirm those of Derbyshire, Smith and Achola (1987), discussed above, and those of Chraemmer-Jorgensen et al (1986) who found no beneficial effect of intravenous lignocaine (1.5mg/kg) given 2 minutes before rapid sequence laryngoscopy and intubation.

Venus et al (1984) reported significantly greater cardiovascular stability after laryngoscopy and intubation in patients pretreated with aerosolized lignocaine (240mg) than in a control group who were given aerosolized saline. In this study laryngoscopy was prolonged for 60 seconds to provide maximal stimulus. The only differences between the Laurito et al (1988) study and the Venus et al (1984) study was that Venus et al (1984) gave their patients a narcotic premedication (morphine 1mg/10kg).

Other authors have reported varying degrees of success with topical lignocaine. Kautto and Heinonen (1982) compared control effects with the blunting effects of pre-induction viscous lignocaine gargle or aerosolized lignocaine. They reported that topical anaesthesia of the upper airway resulting from either viscous lignocaine or lignocaine aerosol prior to induction of anaesthesia, attenuated the magnitude of the pressor response to

laryngoscopy and intubation, but had no effect on the heart rate response. These results confirmed those of Stoelting (1977). Although lignocaine aerosol does not appear to affect the heart rate, there are some potential advantages of the aerosol over the viscous lignocaine. These are a significantly smaller haemodynamic response to the local aneasthetic procedure itself, a shorter duration of the haemodynamic changes caused by the laryngoscopy and intubation, and a lower rate of ECG abnormalities during the procedure (Kautto and Heinonen, 1982). Abou-Madi et al (1975) used a nebulized solution of viscous and aqueous lignocaine prior to induction of anaesthesia. The average measured blood level of lignocaine during intubation in their patients was 1.4 micrograms/ml. They concluded that the inhalation of aerosolized lignocaine provided a highly significant degree of protection during laryngoscopy, but less convincing although still significant protection, after intubation.

There are workers who have preferred to give lignocaine intravenously before laryngoscopy and intubation, probably because of its theoretical advantages of suppression of the cough reflex (Steinhaus and Gaskin, 1963; Poulton and James, 1979), preventing increases in intracranial pressure (Donegan and Bedford, 1980), attenuating circulatory responses (Hamil et al, 1981) and its antiarrhythmic properties (Collinsworth et al, 1974). Abou-Madi et al (1977) compared two dosages of iv lignocaine (0.75mg/kg and 1.5mg/kg) and showed that the higher

dose was more effective in attenuating circulatory responses to laryngoscopy and intubation. Tam et al (1987) reported that a lignocaine bolus iv (1.5mg/kg) given 3 minutes before intubation, produced a statistically significant blunting of the cardiovascular responses. Blood levels of 3.2 +/- 0.6(SE) micrograms/ml have been observed 1.5 minutes after a 1.5mg/kg bolus iv (Bedford et al, 1980). Lignocaine blood levels of between 3 and 6 micrograms/ml are known to potentiate the effects of nitrous oxide anaesthesia in humans and a 10% - 28% reduction in halothane MAC (Minimum Alveolar Concentration) has been reported in dogs with blood levels between 3 and 10 micrograms/ml (Himes et al, 1977). It thus appears that iv lignocaine prevents cardiovascular stimulation at least partially by causing an increase in the depth of general anaesthesia.

Lignocaine given intravenously has been shown to be effective in preventing or reducing the increases in intracranial pressure seen with laryngoscopy and intubation (Poulton and James, 1979; Donegan and Bedford, 1980). Lignocaine causes a 10% - 27% reduction in cerebral metabolic rate for oxygen, and a similar reduction in cerebral blood flow when given to dogs in doses of 3 -15 mg/kg (Sakabe et al, 1975). The suggestion has been made that lignocaine prevents intracranial hypertension by increasing intravascular resistance and decreasing cerebral blood volume, presumably on the basis of reduced local pH, during episodes of cardiovascular stimulation (Hamil et al, 1981). In their study of 22 patients with brain tumours who were undergoing surgery, Hamil and colleagues (1981) showed that topical laryngotracheal

administration of lignocaine did not protect against the cardiovascular or intracranial effects of laryngoscopy and intubation. In contrast, an iv bolus of lignocaine (1.5mg/kg), given one minute before intubation, prevented intracranial hypertension and limited the intensity and duration of the cardiovascular response. In their study the laryngoscopy was begun one minute after the instillation of the 4% lignocaine and the authors comment (but do not substantiate) that in their experience topical anaesthesia is at its maximum 60 seconds after the instillation. Viegas and Stoelting (1975) have demonstrated that blood levels of lignocaine are low one minute after laryngotracheal lignocaine spray, but that they gradually rise to a peak level of 1 - 2.7 microgram/ml between 4 and 15 minutes thereafter. This indicates that laryngotracheal lignocaine is most likely to exert its effect by systemic absorption and not via a direct local anaesthetic effect.

The inconsistant results reported with the use of lignocaine, via any of the described routes, may be explained on the basis of the uncontrolled methodology utilised in the gathering of the data in

most series. In these studies the patients received different premedication including benzodiazepines, and/or narcotics, and in most cases there was an inconsistant approach to the stimulus duration which was usually uncontrolled. The recent reports by Chraemmer-Jorgensen et al (1986), Derbyshire, Smith and Achola (1987), and Laurito et al (1988) do however lend convincing weight to the opinion that lignocaine has little effect on the cardiovascular responses to laryngoscopy and intubation in anaesthetised adults.

1.1.1.4.6.3 Others

1.1.1.4.6.3.1 Labetalol

The use of labetalol, an agent that has non-selective beta adrenergic and selective alpha adrenergic blocking properties, has been advocated by some authors (Leslie et al, 1987; Inada et al, 1987; Bernstein et al, 1987). Although the drug effectively blocks the tachycardia associated with laryngoscopy and intubation, the doses required to attenuate the pressor response are high and may predispose to prolonged hypotension. The alpha:beta ratio of potency is 1:7 and it is therefore not surprising that labetalol will block the heart rate and not the pressor response at low dosages (Chung et al, 1988). Work is

still underway with this drug, but as yet there are no reports of its use in neonates.

1.1.1.4.6.3.2 Fentanyl

Fentanyl, used in massive doses, has been shown to attenuate intubation responses (Stoelting et al, 1975; Stanley and Webster, 1978). Kautto (1982) showed that fentanyl in a dosage of 6 micrograms/kg significantly decreased the heart rate, arterial pressure and rate/pressure product following laryngoscopy and intubation in adults. There is no data available for neonates, but the significant dose-dependant respiratory depression caused by this drug (Downes et al, 1967) would seem to preclude its general use in neonates

1.1.1.4.6.3.3 Vasodilators

Potent vasodilating drugs have been used to attenuate the cardiovascular responses to laryngoscopy and intubation. Hydralazine (Davies et al 1981), nitroprusside (Stoelting, 1979) and phentolamine (DeVault et al, 1960) have all been studied and although there are potent antihypertensive effects, they have no heart rate reducing action. In addition, the use of such drugs is only safe with invasive monitoring, and this is not always

justified. There is no data with regard to the use of these agents in neonates.

1.1.1.4.6.3.4 Ganglion blockade

Siedlecki (1975) showed that the intravenous infusion of trimethaphan (Arfonad) completely prevented any increase in arterial pressure during laryngoscopy and intubation. The sympathetic blockade was attained by an intravenous infusion of 0.1% solution of trimethaphan, beginning before initiation of the general anaesthetic.

1.1.2 Conceptualization and Design of the Study.

1.1.2.1 Phase 1

The pilot study (Phase 1) was designed to test the hypothesis that laryngoscopy and intubation induce changes in the cardiovascular and cerebrovascular systems capable of producing intracranial injury. The purpose of this phase 1 study was to justify the initiation of a second more detailed study of the cerebrovascular effects of laryngoscopy and intubation. The end point of the phase 1 study was to demonstrate a difference in the cardiovascular and histological findings between the two

groups that could be attributed to the experimental stimulus. Because of the expense and the ethical considerations the number of animals was kept to a minimum.

1.1.2.2 Phase 2

The phase 2 study was the first occasion that cerebral blood flow meaurements using radiolabelled microspheres were attempted in this laboratory. The technique observed in the cerebral blood flow laboratory at the University of Pennsylvania School of Medicine was reproduced as closely as possible. The aim in this phase was to set up the laboratory, test the technique and validate the results obtained. Once this was accomplished the technique was to be used to assess the cerebral blood flow changes induced by laryngoscopy/intubation in two groups of pretreated animals - a group in which the larynx and pharynx had been sprayed with saline, and a group in which the larynx and pharynx had been sprayed with lignocaine. The end point of this study was to investigate any differences in the cardiovascular and cerebrovascular responses to the stimulus, and to attempt to assess any differences noted in the Lignocaine group in terms of their cerebral protective potential.

1.1.2.3 Phase 3

Phase 3 was designed as a longitudenal study to observe the effects of laryngoscopy/intubation on regional cerebral blood flow. The phase 2 study provided an indication of the immediate regional blood flow changes during laryngoscopy/inyubation, and phase 3 was intended to add to this knowledge by providing a more complete picture of the changes induced by laryngoscopy/ intubation. Six radio-labelled microsphere tracers were used to allow as detailed a follow-up as possible on the long term changes occurring in the first 20 minutes after intubation. The phase 2 study did not allow demonstration of recovery changes and time intervals, and this led to a situation in which precise deduction of the detailed effects was difficult. Because of the restrictions inherent in the microsphere method of cerebral blood flow measurement viz the limited number of microsphere injections possible, it was decided to spray the cords with lignocaine before the baseline estimation in the Lignocaine group, and to perform 6 estimations thereafter (at the same timepoints in each group) in order to simplify the statistical analysis.

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1 EXPERIMENTAL MODEL, MATERIALS AND METHODS

1.1 EXPERIMENTAL MODEL

1.1.1 Landrace/Large White piglet Hybrid

The animals used in this series of experiments were a hybrid breed of Landrace and Large White pigs. This strain has been shown to be hardy and commercially viable. In addition it is known that these pigs are not of the so-called "hotpig" variety used in studies on malignant hyperthermia.

The selection of the animals is discussed below under animal selection.

1.1.2 Advantages of the model

There were several advantages to using this animal model. On a physical basis, the ease with which the animals could be obtained, the hardy nature of the animal itself, and the fact that the laboratory is set up for animal experimentation with this species were all advantageous.

The newborn piglet has the equivalent neurodevelopmental stage of a 37 week human neonate (Dobbing and Sands, 1979; Pon and Haupt, 1978) and this is important because of the circumstances that stimulated my interest in this field. The number of neonates that

require endotracheal intubation in our situation is higher in term babies than in preterm infants (Groote Schuur Hospital Medical Informatics), and the frequency with which the procedure is used in term babies justifies an investigation specifically into the effects in this group of neonates. Laryngoscopy and intubation are performed daily in the resuscitation of term babies born with asphyxia neonatorum, with meconium aspiration, and with respiratory depression due to drug effects. The effects of raised cerebral flow and increased cerebral pressure resulting from laryngoscopy and intubation have been partially addressed in preterm infants but there remains a dearth of information on the detailed effects in term babies.

1.1.3 Previous use in neurohistologic studies

The newborn piglet model has not been used in any neurohistologic studies of the effects of endotracheal intubation in neonates. This is perhaps due to the fact that most researchers in this field have concerned themselves with the study of periventricular bleeding in preterm animals, and the piglet model, because of its advanced stage of neurodevelopment at birth, is not a suitable model for such work. It appears that there is minimal germinal matrix in term neonatal piglets (Pon and Haupt, 1978) and histologic examination carried out during this series of studies has confirmed this. Since the germinal matrix has been shown to be the principle area of haemorrhage in studies involving preterm

animals (Ment et al, 1982), newborn piglets have been deemed an unsuitable histologic model for the study of periventricular bleeds.
A computer search has not revealed any references in the Medline database.

1.1.4 Previous use in brain blood flow studies

This animal model is well established in a number of research centres as the model of choice for the study of regional brain blood flow changes. Studies on the changes induced by sympathetic stimulation (Busija et al, 1985), hypercarbia (Hansen et al, 1984), hypertension (Brubakk et al, 1984), hypoxaemia (Laptook, 1985), asphyxia (Laptook et al, 1982), and many more stimuli have proven the acceptability of this animal model.

1.2 MATERIALS

1.2.1 Anaesthetic agents

1.2.1.1 Halothane

1.2.1.1.1 Advantages and disadvantages

Halothane (Hoechst) was used as the induction and maintainance agent for anaesthesia despite the fact that it is not the ideal anaesthetic agent for studies involving cerebral blood flow and sympathetic stimulation. There are theoretical disadvantages which include an increase in cerebral blood flow and intracranial pressure, depression of laryngeal and pharyngeal reflexes and a decrease in catecholamine release. In addition the drug is a respiratory and myocardial depressant and increases the incidence of cardiac dysrhythmias (Atkinson et al, 1982).

Although halothane has been reported to sensitize the heart to the arrhythmogenic effect of catecholamines (Katz and Katz, 1966), Lindgren and Saarnivaara (1985) did not see this in their study of the effects of inhalational induction of anaesthesia in children. In fact, Lindgren (1981) reported that the Q-T interval is shortened during the administration of halothane in children. However, he goes on to state that should the Q-T interval be prolonged before the administration of halothane, there may well

be serious ventricular arrhythmias induced by its use. Halothane decreases the firing rate of the sino-atrial node (Flacke and Alper, 1962; Hauswirth and Schaer, 1967).

In spite of the above theoretical disadvantages halothane was found to best suit the requirements for this particular study for the following reasons.

The protocol called for the animals to breathe spontaneously and be capable of maintaining an adequate airway during anaesthesia. The anaesthetic drug should allow the animal to maintain a stable blood gas status and avoid ventilatory support. At the same time analgesia was needed during the insertion of the catheters, and although not a potent analgesic, halothane provided sufficient analgesia to obviate the need for other agents that may have affected cerebral haemodynamics.

In addition halothane has further potential advantages including the fact that it does not cause increased salivation, that there is an element of muscle relaxation associated with its use, and when used in a high flow open system the concentration of delivered gas can be accurately assessed (Atkinson et al, 1982). End-tidal concentration of halothane has been shown to approximate 0.8% after 3 minutes of halothane administration at an inspired concentration of 2% (Lindgren, 1981), and this allows rapid induction with a minimum of stress for the animal. The minimum alveolar concentration (MAC) of halothane for neonates

has been shown to be about 25% less than that in infants (Lerman et al, 1983) implying more pronounced effects of the drug in the newborn (Nicodemus et al, 1969).

Halothane has been shown to depress the laryngeal response to laryngoscopy and intubation (Yakaitis, Blitt and Angiulo, 1977; Lindgren and Saarnivaara, 1985) and in addition it inhibits the burst of efferent sympathetic activity caused by tracheal intubation (Deutsch et al, 1962); any response noted to laryngoscopy and intubation could therefore be expected to be less than would be seen in the unanaesthetised state. This aspect of the study design can be seen to add to the possibility of type 2 (false negatives) statistical errors, but this was deemed acceptable because of the other perceived advantages of using the drug.

Halothane is a known cerebral vasodilator (Atkinson et al, 1982; Christensen et al, 1967; Wollman et al, 1964). In monkeys cerebral vasodilatation does not occur until inspired concentrations of 1.5% (Morita et al, 1977), and in human subjects cerebral blood flow is increased at concentrations ranging from 1.5 to 2 MAC (Fink and Haschke, 1973). Christensen and colleagues (1967) found an average increase in cerebral blood flow of 27% during halothane anaesthesia in normotensive/ normocapnic adults. Wollman and colleagues (1964) reported an increase of only 14%. McHenry and associates (1965) studied a

group of adults during halothane anaesthesia (1%) in combination with nitrous oxide and oxygen, and demonstrated a 55% increase in cerebral blood flow. This result was probably influenced by the moderate degree of hypercapnia (PaCo2 = 47 mmHg) that was seen in these patients.

Halothane has thus been shown to increase cerebral blood flow, but has less of an effect on the cerebral vasculature than nitrous oxide (Hansen et al, 1988; Samra et al, 1988) which was avoided for this reason. The addition of nitrous oxide to a volatile anaesthetic background (halothane or isoflurane) has been shown to increase the cerebral blood flow by a greater degree than would be obtained by simply increasing the concentration of the volatile agent (Hansen et al, 1988). Samra and colleagues (1988) showed that nitrous oxide increased cerebral blood flow in a dose dependant manner, but that this increase was differential in terms of an anterior-posterior gradient. This increase is different from the increase produced by simple vasodilatory agents like CO2 which cause a uniform increase in cerebral blood flow in all cortical areas.

One of the major motivations for using halothane in this study was the fact that many babies requiring emergency intubation have been delivered by caesarean section. In our institution (Groote Schuur Hospital) there is a high incidence of eclampsia, and because of its epileptogenic properties enflurane is avoided. Halothane is thus the volatile agent of choice and neonates delivered by caesarean section would have been exposed to

halothane in utero. Biehl et al (1980) studied the uptake of halothane in the fetal lamb in utero, and found that the uptake in the fetus parallels that of the mother. Within 2 minutes of maternal exposure to halothane the fetal level was found to be 36% of the mother's level, and by 64 minutes of exposure the fetal level was 76% of its mother's. There was significant decrease in fetal blood pressure after only 8 minutes of maternal exposure indicating that despite the lower concentration the effects are still significant. This is possibly related to the greater potency of the drug in the neonate (Lerman et al, 1983).

Ultimately there is no ideal anaesthetic agent for a study of this nature where cerebral blood flow and sympathoadrenal outflow need to be assessed. However halothane has been shown to be suitable for the anaesthetising of small animals (Wagerle and Delivoria-Papadopoulos, 1987), and has been shown to have less disturbing influences on the cerebral blood flow than nitrous oxide (Hansen et al, 1988). With the clinical applicability in mind this drug was chosen as the anaesthetic agent for this study.

1.2.1.2 Experience with other agents

1.2.1.2.1 Rejection of thiopentone

The barbiturate anaesthetic agents were not used because of their cerebral stabilizing properties which include reduction in cerebral blood flow (Pierce et al, 1962) and cerebrospinal fluid pressure, decreased intracranial pressure, decreased cerebral oxygen consumption (Atkinson et al, 1982), and, at least in primates, a reduced degree of cerebral damage following global ischaemia (Bleyaert et al, 1978). In addition the respiratory depression and hypersensitivity of the larynx to any form of stimulation caused by these agents precludes adequate spontaneous respiration.

Another major consideration was the anticipated vasoconstrictor effect of laryngoscopy/intubation on the cerebral vasculature. The use of an anaesthetic that had a cerebral protective or vasoconstrictor effect would have interfered with and possibly masked any experimentally induced effect.

The use of barbiturate agents in small animals is complicated by the fact that the drug is difficult to administer and is usually given intermittantly into the peritoneum. The depth of anaesthesia is not constant and this may lead to an erratic anaesthetic with changes in haemodynamic parameters that interfere with the experimental protocol.

1.2.1.2.2 Rejection of ketamine

Ketamine is a cerebral vasodilator (Dawson et al, 1971; Takeshita et al, 1972) and has been shown to cause up to a 200% increase in cerebral blood flow. In addition there is a pressor cardiovascular effect (Atkinson, Rushman and Lee, 1982). For these reasons, as well as because of the difficulty with administration and maintenence of the anaesthesia in neonatal animals (excitement and shivering), this agent was rejected.

1.2.1.3 Spontaneous ventilation

There were many reasons for using a spontaneously breathing animal model for this study rather than a tracheostomised, artificially ventilated model.

The stimulation of performing the tracheostomy required for ventilation, and the subsequent constant stimulation of the endotracheal tube on the tracheo bronchial tree, would provide an uncontrolled source of irritation capable of affecting the measured parameters. This was the major reason for choosing a spontaneously respiring model despite the obvious difficulties inherent in such a model.

In addition the possible pressure effects of ventilation on both the cardiovascular and cerebral blood flow measurements would confuse the subsequent analysis. Mechanical ventilation requires paralysis of the animal and necessitates the use of drugs with

possible haemodynamic effects. Finally, the clinical situation of performing laryngoscopy and endotracheal intubation on a tracheostomised patient is rare.

The disadvantages of using a spontaneously respiring animal model relate to the maintanence of an adequate airway, and the maintanence of a smooth anaesthetic. Because of this it was expected that there would be a certain degree of rebreathing and that the animals would be mildly hypercapnic. This was however acceptable in terms of the clinical situation in which the results of this study were to be applied. A healthy well oxygenated neonate rarely requires ventilation in the first few hours of life, and this study is rather aimed at the baby with some degree of hypercapnia and respiratory depression.

1.2.1.4 Oxygen

Oxygen was used as the carrier gas during anaesthesia. In retrospect the choice of 100% oxygen as the delivery gas was not the ideal in view of the hyperoxia which was encountered. There are however studies involving the determination of regional cerebral blood flow in which the animals have been ventilated with 70-80% oxygen (Wagerle and Delivoria-Papadopoulos, 1987; Kurth, Wagerle and Delivoria-Papadopoulos, 1986). The rationale for the use of 100% oxygen in the present study was to avoid hypoxia because of its cerebral vasodilating and myocardial

depressing effects, both of which would mask and confuse the responses to the intended stimulus. The bradycardia usually noted with laryngoscopy and intubation in neonates in most series is almost always accompanied by some degree of hypoxia. It was argued that by providing 100% oxygen to animals spontaneously breathing a respiratory depressant agent, hypoxia would be avoided and that the 10 - 30% decrease in cerebral blood flow (Shinozuka and Nemoto, 1981; Kennedy et al, 1971) resultant upon the high oxygen tension would be offset by the overiding vasodilatory effects of the hypercapnia and halothane. There is evidence in adults that hyperoxia has little or no effect on cerebral blood flow Busija et al, 1988).

In terms of the clinical applicability of this design, most neonates are mask ventilated/hyperventilated with 100% oxygen or given continuous 100% oxygen via a modified laryngoscope blade (oxyscope) (Hinkle, 1986) during the preparation prior to intubation, and thus it would not be uncommon to encounter a certain degree of hyperoxia in an infant with hypercarbia (atelectatic air trapping) in the clinical situation. It has been shown that only 30 seconds of breathing (without deep breaths) 100% oxygen is sufficient to increase the PaO2 from 70 mmHg to 300 mmHg (Heller et al, 1962).

1.2.1.5 Lignocaine

Lignocaine (Remicaine, Labethica) 2% was used as the local anaesthetic agent for laryngeal and vocal cord spraying. A total of 4 puffs of this 2% solution was delivered to the larynx via a modified Forrester spray. This corresponded to a total dosage of 8 mg.

Local anaesthetics have been widely used as anticonvulsants (Berry et al 1961), adjuncts in general anaesthesia (Koppanyi, 1962), analgesics (Keats, 1951) and as antiarrhythmic drugs (Harrison et al, 1963).

The haemodynamic effects of lignocaine have been examined by a number of workers in both humans and experimental animals. Wiklund (1977) noted slight increases in heart rate, mean arterial pressure, and cardiac output in healthy volunteers receiving lignocaine infusions with plasma concentrations of 2.4 micrograms/ml. Klein et al (1968) reported increases in mean arterial pressure and systemic vascular resistance with no change in cardiac output in patients with coronary heart disease or valvular heart disease.

Little, however, is known about its effects on cerebral metabolism and blood flow. Lescanic et al (1981) showed that in laboratory rats there were no significant differences in heart rate, mean arterial pressure, cardiac output or tissue blood flow at blood levels of lignocaine of 1.98 +/- 0.27 micrograms/ml. At a higher blood level (6.37 +/- 0.29

micrograms/ml) there were significant decreases in heart rate and cardiac output, and in tissue blood flow to the brain, heart and muscle. Brain blood flow decreased due to an increase in cerebral vascular resistance. The exact mechanism whereby lignocaine effects this change is unknown, but may serve to explain why lignocaine tends to reduce the increases in intracranial pressure seen when endotracheal suctioning is carried out. Although lignocaine is generally considered to be a vasodilating agent and at high concentrations relaxes all vascular smooth muscle, it is not impossible that under certain circumstances it might constrict some vascular beds. Lignocaine has been shown in vitro to increase basal tone and spontaneous contractions in isolated rat portal vein (Klein et al, 1968). It also initially enhances contraction of arterioles in rat mesocaecum induced by catecholamines (Altura, 1967). Hyman (1970) has shown that in dogs lignocaine can cause pulmonary venous constriction in vivo, while Cibils (1976) demonstrated that lignocaine caused in vitro constriction of human uterine arteries taken from pregnant women. Obviously the direct extrapolation of the results of animal experiments to humans is invalid, but this type of research does provide possible explanations for observed phenomena.

1.2.2 Anaesthetic Equipment

1.2.2.1 Anaesthetic Machine

An anaesthetic machine was designed and built for this study (See plate .). It has the specific capability of delivering 4 gases simultaneously viz oxygen, nitrous oxide, nitrogen, and carbon dioxide. In addition there is a Fluotec Mk.3 halothane vapourizer (Cyprane Ltd) in circuit. The system could be used as a closed circuit with a circle carbon dioxide absorber or as an open circuit with a modified Ayre's T-piece (Ayre, 1967) system attached to a head-box. There was an oxygen analyser connected at the combined gas outflow point.
The apparatus was built and tested by the technical division of the Dept. of Anaesthetics at Groote Schuur Hospital.

1.2.2.2 Head Box

A standard perspex neonatal head-box was used as the face mask for anaesthetising the animals. This was connected to a modified Ayre's T-piece (Ayre, 1967) gas delivery circuit and effectively formed a Mapleson-E circuit. The opening and base of the box were sealed with latex rubber strips. The opening was closed off in such a way as to allow an airtight seal around the animal's

muzzle. A canister of Wilson soda-lime was placed within the head -box for carbon dioxide absorption.

1.2.2.3 Carbon Dioxide Absorber

A circle-system carbon dioxide absorber was included in the original anaesthetic machine to be used during mechanical ventilation if required, but this facility was not needed in this set of experiments. Fresh gas supply rate was calculated to safely exceed animal minute ventilation and minimum anaesthetic circuit requirements to prevent carbon dioxide rebreathing. Nevertheless a canister of soda-lime was placed in the head-box to further guard against hypercarbia.

1.2.2.4 Laryngoscope

A standard neonatal straight laryngoscope with a 10cm blade was used in all cases.

1.2.2.5 Cord Aerosol Spray

A modified Forrester spray was used for anaesthetising the larynx (Forrester, 1974). The modification consisted of replacing the spray tubing with tubing of a smaller diameter. The detailed

technique of its use is described in the relevent section. The apparatus delivered a volume of 0.1ml per spray.

1.2.2.6 Endotracheal Tubes

A shouldered Portex neonatal endotracheal tube was used in all cases. The tube diameter was 2mm - 3.5mm and the length 12cm. A removable plastic stylet was placed inside the tube to maintain the rigidity of the tube during intubation.

1.2.3 Data Recording System

1.2.3.1 Computer System

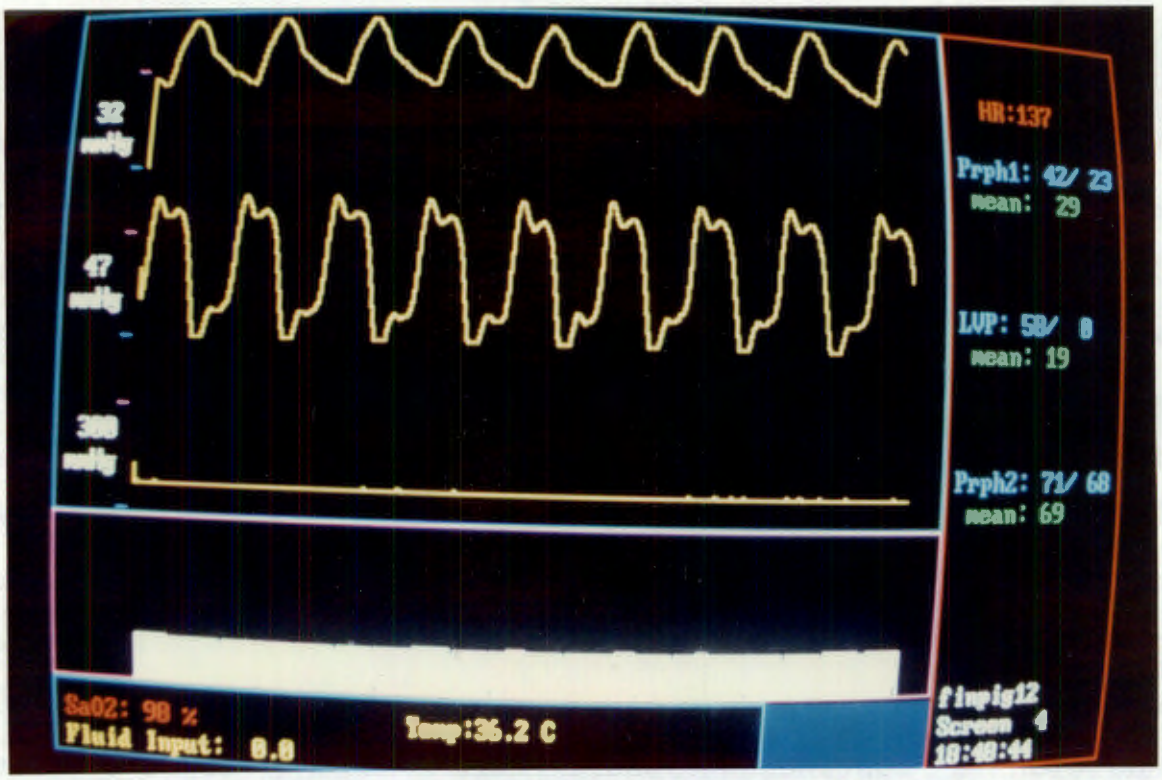
The data recording system was designed and developed specifically for this study. This integrated computerised data acquisition system allowed accurate and meaningful analysis of the collected data, and the trending facility enabled correlation and comparison of data on a level not previously attainable.

The basic equipment consisted of a 40 megabyte hard disk computer (M & PD, Mercedes Datacor) with a single 360 kilobyte floppy disk drive. A mathematics co-processor (80872), an analogue-to-digital board, and an Enhanced IBM Graphics card were added to the system. The program was designed by the author and the software

was written by Mr. Raymond Parfitt of the Department of Biomedical Engineering.

1.2.3.2 Data Acquisition Program

The data acquisition program allowed simultaneous acquisition and display of data from all of the monitors used during the experiment. This included a Mennen Greatbatch monitor (2X pressure tranducers, respiratory rate, and temperature), a Camino monitor (1X System 420 fibre optic pressure tranducer), and a Nellcor peripheral pulse oximeter. Because of the difficulty in obtaining a consistent, technically acceptable ECG signal, the heart rate was calculated from the left ventricular pressure trace by the computer and continuously displayed on the monitor. In addition a continuous short term trend of the left ventricular pressure was displayed in order to facilitate the insertion of the left ventricular catheter. Using this trend the change in diastolic pressure on entering the ventricle was graphically displayed and accompanied by an audio signal.
The program allowed storage of up to 100 screens per animal and this ensured that dysrythmias or other perturbations were recorded with all parameters simultaneously displayed.



Screen display

The analogue signals from the monitors were saved for a period of 10 seconds and the mean value was then stored to disk. This allowed analysis of the data to a sensitivity of 10 seconds at any time point in the study. A comments file was included for the storage of blood results and other information.
During the experiment the exact times of certain events were recorded using marker symbols. A timer was included for procedures that required timed blood collection eg the withdrawal of the reference blood specimen in microsphere experiments.

An example of the screen display is shown on the opposite page.

1.2.3.3 Data Trending Program

The data trending program allows graphical display of all recorded parameters over periods ranging from 10 seconds to 4 hours. In this study the time periods set were 5 minutes, 20 minutes, 1 hour, and 4 hours. As seen in some of the examples below, the time of specific events is marked on the x-axis.

1.2.3.4 Data Storage

All data was stored primarily on the hard disk but was transferred to floppy disks once the experiment was completed.

1.2.4 Monitoring Equipment

1.2.4.1 Arterial Pressure

All fluid tranduced pressures were carried out using a Mennen Greatbatch (Marcus Medical) monitor. The transducers were solid state non-disposable P50 transducers with disposable Gould P50 transducer domes.

1.2.4.2 Brain Tissue Pressure and CSF pressure

Brain tissue pressure and cerebrospinal fluid pressure were recorded using a Camino System 420 fibre-optic tranducer-tipped catheter system. Flushing is not required to maintain accurate readings with these solid state catheters, but one disadvantage is that they do not allow the withdrawal of blood specimens.

1.2.4.3 Peripheral Oxygen Saturation

A Nellcor peripheral pulse oximeter was used to record the brachial artery pulse and provide a continuos peripheral oxygen saturation level. A non disposable fibreoptic sensor was used.

1.2.4.4 Temperature Probe

A non disposable Mennen Greatbatch rectal temperature probe was used and connected to the Mennen Greatbatch monitor.

1.2.4.5 Electrocardiogram

In phase 1 an electrocardiographic trace was recorded using the Mennen Greatbatch monitor. The technical difficulty in achieving an adequate trace precluded further use of the ECG for heart rate determination, and the computer generated heart rate was used for all successive phases.

1.2.5 Radioactive Microsphere Equipment

1.2.5.1 Microspheres

All microspheres were obtained from Separation Scientific an agent of New England Nuclear, Boston, MA (DUPONT). The following tracer microspheres were used at indicated times during the study.

Name	Half-life	Energy	Photon Abundance
Cobolt-57	271 days	122-136 KeV	98%
Cerium-141	32.5 days	145 KeV	48%
Chromium-51	27.8 days	320 KeV	9%
Tin-113	115 days	393 KeV	64%
		255 KeV	2%
Ruthenium-103	39.8 days	497 KeV	88%
		610 KeV	6%
Niobium-95	34.9 days	765 KeV	98%

Scandium-46	84 days	889 KeV	100%
		1.12 MeV	100%

The microspheres were all 15 micron in diameter and were supplied in 10ml 0.9% saline with 0.01% TWEEN-80.

1.2.5.2 Injection Vials

Injection vials were stock of the laboraratory at the Department of Physiology, University of Pennsylvania, Philadelphia and were supplied courtesy of Professor L. Craig Wagerle. These vials were hand blown according to specifications and following the description in the article by Heymann (Heymann et al, 1977).

1.2.5.3 Harvard Pump

The withdrawal pump was a standard Harvard pump (Harvard Apparatus, Millis, MA).

1.2.5.4 Withdrawal Equipment

The accessory apparatus for reference blood withdrawal included:
10ml glass syringes

Heparinised PE50 tubing

3-way tap

1.3 METHODOLOGY

1.3.1 Introduction

1.3.1.1 Outline

The division of this study into 3 consecutive, separate, experimental phases has made the description of the experimental methodology potentially repetitive. For this reason methods that were employed in all 3 phases are described in the general introduction. Where procedures or tests specific to the experiment under consideration were performed, a full description of the methods employed is made in the relevant section.

Phase 1 - The phase 1 experiment was designed to investigate the cardiovascular and histologic changes following laryngoscopy and intubation. There were two groups of piglets, a Control group (n = 6) that underwent anaesthesia and monitoring, and an Intubation group (n = 11) that had anaesthesia, monitoring and laryngoscopy/ intubation.

Phase 2 - The phase 2 experiment was designed as a totally separate experiment with the main objectives being measuring cardiovascular changes during laryngoscopy/intubation, and the study of the brain blood flow changes following laryngoscopy/ intubation, with and without prior spraying of the larynx and pharynx with lignocaine. There were two groups of animals, a Saline-spray group (n = 10) and a Lignocaine-spray group (n = 10).

Phase 3 - The phase 3 experiment was designed to further investigate the cardiovascular changes and to broaden the picture of the cerebrovascular changes demonstrated in phase 2, following laryngoscopy/intubation. There was a Control group (n = 6) and a Lignocaine group (n = 6).

The detailed methodology of these 3 separate experiments is described below.

1.3.1.2 PHASE 1

1.3.1.2.1 Experimental detail

Animals were randomised from a computer generated table into two groups. The Control group was limited to the minimum number of animals regarded as statistically representative (6 animals). The experimental group was limited to double this number. Detail of all monitoring procedures is provided elsewhere in the text.

The Control group (n=6) underwent anaesthesia and insertion of monitoring equipment. This included an umbilical artery pressure catheter (Camino), a second umbilical artery catheter for blood specimen withdrawal, a Camino interstitial brain tissue pressure monitoring catheter (n=2), and a rectal temperature probe. These animals were then allowed to stabilize for 10 -15 minutes and had baseline monitoring procedures (electrocardiographic, rectal temperature, brain tissue pressure, blood pressure and blood gases) carried out at the end of this period. The Control piglets were then removed from the head box and held with their heads in the same way that the animals undergoing laryngoscopy and intubation were held. This position ("sham intubation") was maintained for a period of 3 minutes. The animal was then replaced in the head box and monitoring continued for the same length of time as in the Intubation group. At the end of the experiment the monitoring equipment was removed. The abdominal wound was closed leaving one umbilical artery catheter in-situ.

The piglet was then removed from the table and placed in a warmed recovery area. Three to four hours after removal from the operating table the animals were sacrificed by an intra-arterial injection of thiopentone sodium (Intraval, May and Baker).

A second group (n=12) were anaesthetised and monitored in the same way. One of these animals died soon after the induction of anaesthesia, before laryngoscopy/intubation, and was excluded from the results. In nine of these animals brain interstitial tissue pressure monitoring catheters were sited. Following stabilization and baseline recordings, the animals were removed from the head box, direct laryngoscopy was performed for a period of one minute and the vocal cords were visualised. It must be stressed that the animals did not struggle during this procedure and were still very drowsy and apparently unaware. There was certainly no indication of extreme discomfort or distress even when the larynx and vocal cords were irritated, in the manner described above, and none of the animals coughed. Following this the trachea was intubated with a 3mm Portex neonatal endotracheal tube. This procedure was carried out by the same investigator on all occasions in an attempt to standardise the insult. Changes in the mean arterial pressure (MAP), blood gases and brain tissue pressure were recorded immediately following the intubation period. The endotracheal tube was left in situ for a period of 2 minutes after which time it was removed. The animals were then re-anaesthetised and managed as described above with sacrifice occurring 3 - 4 hours after removal from the operating table.

The reason for designing this study in this way was influenced by an attempt to recreate the clinical situation. In the resuscitation of an asphyxiated neonate delivered by caesarean section, there would usually be a delay of a few minutes from the time of cord clamping (arresting exposure to the halothane), during which time "bagging" with 100% oxygen would be instituted prior to the laryngoscopy and endotracheal intubation.
A 1 minute period of laryngotracheal stimulation was chosen to maximise the response. This duration of insult may appear excessive to an expert clinician, but it is not unusual in the emergency clinical situation in hospitals where the majority of neonatal resuscitations are performed by non-specialised, and often junior staff, and prolonged laryngoscopy with repeated attempts at intubation is not infrequently witnessed.

In a study of this nature it is extremely difficult to ensure that each animal has exactly the same stimulus. As far as possible each step of the protocol was controlled to provide equal experimental conditions. The possibility that some of the monitoring procedures would be responsible for intracerebral bleeding was considered, and so as to assess the effect of the BTP transducer in both groups, not all of the animals in each group had the transducer inserted.

1.3.1.2.2 Blood specimens

1.3.1.2.2.1 Arterial blood gases

Arterial blood gas estimations were carried out at the time of the baseline readings, and again immediately post intubation. The technique has been described above.

1.3.1.2.2.2 Transfusion/Fluid replacement

Since only small amounts of blood were routinely removed, blood was not replaced unless there had been difficulty during catheter insertion. Fluid replacement was with 0.9% saline.

1.3.1.2.3 Statistical analysis

The data are reported as means +/- standard deviation. The Mean Arterial Pressure (MAP), pH, PaCo2, base excess and brain tissue pressure were analysed using a one-way analysis of variance with a 5% level of significance. The histologic data were analysed using Fischer's Exact Test.

1.3.1.3 PHASES 2 AND 3

1.3.1.3.1 Experimental detail

1.3.1.3.1.1 Animal preparation

Newborn Landrace/Large White hybrid piglets were used for this study. As with phase 1, all were removed from the teat at less than 6 hours of age having been born on the morning of the study.

Induction of anaesthesia was carried out as detailed above. Once asleep the animal was transferred to the operating table and the modified head-box gas delivery system was placed over its muzzle and face. A container of carbon dioxide absorbing soda-lime crystals was left inside the head-box. The piglet was taped to the table in a supine position with its neck extended, and the adequacy of its airway was ensured.

A rectal temperature probe was inserted and connected to the monitor (Mennen Greatbatch). A warmed air source heater was directed over the animal and allowed regulation of the animal's body temperature within a range from 37 to 39 degrees celcius.

A Nellcor N-100 pulse oximeter was placed on the right forelimb to monitor peripheral oxygen saturation and heart rate.

In the first ten animals entered into the phase 2 study arterial catheter placement was via the femoral vessels. In the remaining animals (in both phase 2 and phase 3) the umbilical vessels were used. The insertion techniques are described below.

A stabilization period was then allowed.

1.3.1.3.1.2 Study design

1.3.1.3.1.2.1 Phase 2

Animals were randomized into two groups using a computer generated table. The two groups will be referred to as the Saline-spray group (n=10) and the Lignocaine-spray group (n=10). An assistant prepared and "blinded" the spray solution so that the principle investigator was unaware as to the nature of the solution in the Forrester spray. The code was broken at the end of the study.

Both groups underwent identical preparation as discussed above. A stabilization period was allowed as described below. Following withdrawal of baseline arterial blood gas specimens the animals were prepared for the baseline microsphere injection (Strontium-85). Preparation of the stock microsphere solutions and the detailed technique of injection and withdrawal have been described above.

Following baseline microsphere injection a 5 minute period was allowed for re-stabilization.

Laryngoscopy and laryngeal and pharyngeal spraying was then carried out and 15 minutes after the completion of this procedure the second microsphere tracer, Scandium-46, was injected.

The 15 minute period was allowed for the haemodynamic changes that occurred during laryngoscopy and spraying to settle. Laryngoscopy and endotracheal intubation was then performed by the same investigator in the same manner in all cases. The third microsphere injection, and the collection of the reference blood were made during the latter period of laryngoscopy and overlapped with the period of tracheal irritation. The microsphere injection was thus completed before the extubation, and collection of the reference blood specimen did include part of the 1 minute that the tube was left in-situ. Cerium-141 was used as the laryngoscopy/intubation tracer microsphere. In terms of the timing of the microsphere injection, the blood flow rate measured at the time of injection was that corresponding to the laryngoscopy and immediate post tracheal irritation timepoint. Following the final microsphere injection and blood specimen withdrawal, and once the blood pressure had again reached baseline levels, the animal was removed from the table and further management was as described above. In phase 2 the animals were treated as in the phase 1 histology study with sacrifice occurring at 3-4 hours post removal from the table.

1.3.1.3.1.2.2 Phase 3

Phase 3 was designed as a longitudenal study to observe the effects of intubation on regional cerebral blood flow. Six microsphere injections were used to allow as detailed a follow-up

as possible on the long term changes occuring in the first 20 minutes after intubation. The phase 2 study provided an indication of the regional blood flow changes immediately following laryngoscopy/irritation, but in order to complete the picture and allow a fuller understanding of the underlying mechanisms, a longitudenal study incorporating earlier and later flow rate estimations was required.

The animals were divided into two groups, a Lignocaine group (n=9) and a Control group (n=9). Both groups of animals underwent identical preparation to the piglets in the phase 2 microsphere study.

The Control group were allowed a 15 minute period of stabilization following insertion of monitoring equipment prior to the base line microsphere injection (Cobolt-57). Following this, 15 minutes was allowed for the animals to settle to their baseline haemodynamic levels. At this point laryngoscopy and intubation was carried out and the Chromium-51 microsphere injection was started within 10 seconds after the start of the laryngoscopy, ie at least 40 to 45 seconds before the equivalent estimation in phase 2. Immediately following completion of the reference blood withdrawal, intubation was initiated. The endotracheal tube was removed approximately 2 minutes after intubation, and immediately following this the Tin-113 labelled microspheres were given. Ruthenium-103 microspheres were injected at 7 minutes, Niobium-95 microspheres at 15 minutes and

Scandium-46 microspheres at 20 minutes from the start of the intubation. In this set of experiments the laryngoscopy was timed for 1 minute, the cord probing for 20 seconds and the tube remained in-situ for 2 minutes (see above). Standard laboratory timers were used.

The Lignocaine group had laryngeal and pharyngeal spraying (as described above) at the completion of the catheter insertion and were then allowed a 15 minute period of stabilization before the baseline Cobolt-57 microsphere injection. The experiment was completed in the same way as with the Control group.

Because of the constraints imposed by the limited number of microsphere injections allowed (a maximum of 6 different labels) the design of the experiment has been such that the exact effects of the laryngeal spraying (during the spraying) have not been investigated.

From the results of phase 2 it appeared that there were no significant differences in the baseline haemodynamics or regional brain blood flow rates between the group sprayed with saline and the group sprayed with lignocaine once sufficient time was allowed for settling. Using the data from this study it was assumed that neither the lignocaine, nor the act of spraying the larynx with lignocaine altered the baseline pre-laryngoscopy/ intubation haemodynamics to any significant degree.

In the clinical situation it would be extremely rare that a patient would have laryngeal spraying with a local anaesthetic without subsequent intubation. Investigation of the regional brain blood flow changes induced by laryngeal spraying would be interesting, but of little clinical significance. By designing the experiment in the above way it was possible to analyse the changes in regional cerebral blood flow following laryngoscopy and intubation in the two groups, with the only variable being the fact that one group had been given the drug prior to the insult. The two groups were treated in exactly the same way after the baseline microsphere shoot allowing an easier and more direct analysis of the results.

1.3.2 Animal Supply and Selection

Animals for this study were all supplied from the Simonsberg Piggery. This is a commercial pig farm owned by the Back brothers and is situated in Klapmuts, approximately 50 kilometers from Cape Town. The farm is the official supplier of pigs to the University of Cape Town Medical School Animal Unit. Standards of hygiene are strictly monitored and the farrowing house is of the highest order. Piglets for this study were selected from litters of neonates born within 6hrs preceding the collection time. In most instances 2 piglets were collected at any one time, but on occasion up to 5 animals were taken. The animals were left with the sow up until the moment of collection to allow maximal

feeding opportunity prior to separation. Piglets were selected from a single litter and were as far as possible weight matched. Animals known to be anaemic were excluded. Only Landrace/Large White hybrids were used in this study.

1.3.3 Transport

The animals were removed from the sow and placed in a cardboard container. The umbilical cords were trimmed and the ligatures checked prior to departure. The box with the piglets inside was transported in the passenger compartment of a sedan. The heater was used to prevent hypothermia. On arrival at the laboratory the piglets were placed in a warmed box.

1.3.4 Induction of Anaesthesia

Induction of anaesthesia was accomplished by gently holding the animal and allowing a stream of gas (Halothane 3% in oxygen) to play across the muzzle. In most cases this led to a smooth inhalational induction of anaesthesia. Once the animal was asleep it was secured on the operating table and its muzzle was placed into the modified head box. A container of soda-lime was placed inside the head box.

1.3.5 Position on operating table

The piglets were placed on the operating table in the supine position and their limbs were secured with masking tape. Care was taken to ensure adequate thoracic movement and a good airway.

1.3.6 Maintainance of anaesthesia

The level of anaesthesia was titrated to maintain adequate spontaneous respiration. Continuous peripheral oxygen saturation was monitored (Nellcor N-100 peripheral oximeter). The pulse rate and blood pressure were used as indicators of the depth of anaesthesia. An oxygen flow rate of 6-7 litres per minute and a halothane level of between 1% and 1.5% was found, in most cases, to be sufficient to maintain surgical levels of anaesthesia.

1.3.7 Oxygen Saturation Monitoring

A Nellcor N-100 pulse oximeter was used to continuously monitor peripheral oxygen saturation in phases 2 and 3. The sensor was taped around the right forelimb of the anaesthetised animal.

1.3.21.3 Staining and mounting

The slides were examined independantly by two members of the neuropathology department, Dr. R. Bowen and Mr. C. Gouveia, who were unaware of the study group from which the specimens had come.

1.3.22 Preparation of specimens

1.3.22.1 Regional brain dissection

1.3.22.1.1 Specific areas identified

1.3.22.1.1.1 Preparation

The dissection of the brain in the microsphere studies was much more complex than in the histology studies where brain slices were required. For regional brain blood flow studies the areas in question had to be dissected out as accurately as possible. To achieve as representative a region as possible the brain was again sliced into coronal sections. The slices were positioned at the optic chiasma, at the level of the Trigeminal nerves, at the junction of pons and medulla and at the juction of the medulla and upper cervical spinal cord. By dividing the brain up in this

artery. A bulldog clamp was used proximally to control haemorrhage and traction on the distal ligature used to control back-bleeding. The vessel lumen was then entered. Using a vessel dilator the anterior arterial wall was elevated and a size 3 umbilical artery catheter (Argyle) was introduced into the lumen. The catheter was previously prepared by having the tip twisted and heated to ensure a "pigtail" at the distal end. Before insertion into the vessel a thin wire stylet (Deseret Co.) was inserted into the catheter to facilitate manipulation. The catheter was then advanced into the vessel and connected to the pressure transducer (P50, Gould). It was then further advanced under waveform control until it entered the left ventricle. This was confirmed by a sudden drop in the diastolic pressure displayed on the computer screen, and by a computer generated audio signal that was programmed to sound when the diastolic pressure was less than 6mmHg. Once catheter placement was confirmed the ligature was tied and the catheter flushed with saline (1ml, 0.9%). The incision was then covered with a moist swab.

1.3.9.1.2 Umbilical artery approach

For placement of the left ventricular catheter via the umbilical artery a 2cm subumbilical midline incision was made and the umbilical vessels were exposed. The vessels were dissected free of the bladder and ligatures placed proximally (for traction) and

distally for haemorrhage control. A bulldog clamp was used to prevent blood loss distally while the vessel was entered and the catheter pushed into the lumen. Negotiation of the junction of the umbilical and iliac arteries required a little practise initially, but once this technique was mastered this approach was found to be more satisfactory than the femoral approach. Once the catheter was advanced into the aortic lumen placement of the catheter tip in the left ventricle was accomplished as described above.

Following siting of the catheter (or catheters - see below) the incision was closed with 3/0 polyglycolic acid suture.

1.3.9.2 Brachial Artery

The left brachial artery was used in all cases for placement of the reference blood withdrawal catheter. A 2cm incision over the left humerus was made to expose branches of the brachial plexus and the brachial artery and vein. Utilising an operating microscope the brachial artery and vein were dissected free and ligatures were placed to control bleeding. A bulldog clamp was used to prevent haemorrhage when the vessel lumen was opened and a PE50 polyethylene catheter (Intramedic, Clay Adams Co., Parsippany,NJ) was introduced into and advanced up the brachial artery into the axillary artery (approximately 2cm). Once in

position the catheter was secured by the ligature. Free blood backflow was confirmed and the catheter was flushed with saline (0.9%) and connected via a three way tap to the Harvard Pump.

1.3.9.3 Abdominal Aorta

1.3.9.3.1 Femoral or Umbilical approach

Monitoring of arterial pressure during injection of the microspheres in the blood flow studies was essential. Since microsphere injection was via the left ventricular catheter, a second arterial catheter was required.
In those animals that had the left ventricular catheter inserted via a femoral vessel, the aortic catheter (Camino system 420 transducer tipped catheter) was placed via the other femoral artery, and the same applied for catheter insertion via the umbilical arteries.

1.3.9.3.2 Camino System

In the phase 1 and 2 studies Camino system 420 transducer tipped catheters were used to monitor aortic arterial pressure. These catheters were inserted and fixed in the same manner as described above for the fluid filled catheters. The only difference in

catheter management was that the Camino catheters did not require flushing.

1.3.10 Brain tissue pressure (BTP) catheter

In the phase 1 study brain interstitial tissue pressure was measured using Camino transducer-tipped catheters. Following insertion of all intravascular monitoring catheters the animal's head was removed from the head box and positioned by an assistant so as to expose the right parietal area. A v-shaped incision was made in the scalp and the tissue was reflected to expose the underlying cranial bone. Using a disposable Camino hand-held drill a hole 3mm in diameter was made through the skull bone. A Camino cranial bolt was then screwed into the bone and the dura perforated with the provided dural needle. The Camino catheter was then advanced into the bolt to a point corresponding with cortical penetration to a depth of 3-4mm. The incision was then sutured. The animal's head was replaced in the head-box and 20 minutes allowed for haemodynamic and cerebrovascular stabilisation. Baseline readings were then made following catheter calibration. Because of the small changes and large inter animal variation all readings were taken from a baseline of 0 mmHg. The catheter was calibrated to read 0 mmHg just prior to laryngoscopy/intubation.

1.3.11 Routine Blood Analysis

1.3.11.1 Blood Gases

Blood gas measurements including pH, PaCO2, and PaO2 were performed at different times (as indicated) in each study phase. Heparinised (Pularin, 5000units/ml) 1ml tuberculin syringes were used and 0.2ml of blood was removed from the aortic catheter for each determination. Analysis was carried out using a Radiometer BMS 3 Mark 2 analyzer. Base excess or deficit was calculated using a standard Sigaard-Andersson nomogram.

1.3.11.2 Haematocrit

Haematocrit was determined routinely with the initial blood gas specimen, as well as before and after blood transfusion in those animals who had transfusion (as indicated in the relevant sections). Transfusion was reserved for animals having appreciable blood loss during catheter insertion (>5ml), and all animals who had >5ml of blood removed in the course of the experiment. The arterial haematocrit was calculated by the microcapillary technique using blood from the aortic catheter.

1.3.12 Fluid Replacement

1.3.12.1 Crystalloid

Normal saline was used as the crystalloid replacement solution during this series of experiments. In those animals having an estimated blood loss of less than 5ml crystalloid, rather than blood, was used as a fluid replacement. This was infused as bolus amounts never exceeding 2ml at any one time. In addition all infusate was kept at 37 degrees Celsius in a water bath.

1.3.12.2 Blood

Blood was transfused in those animals having a blood loss in excess of 5ml. Blood was taken from donor pigs and stored in heparinised tubes in a warm (37 degrees celsius) water bath. Fresh blood was prepared for each day of experiments. Transfusion was by slow intra-arterial injection of 5ml amounts over a period of 3-4 minutes. All transfusion was carried out under continuous arterial pressure and heart rate monitoring, and where possible was marked on the computer recording with a specific symbol.

1.3.13 Timing of baseline estimations

Following insertion of the monitoring equipment a period of between 10 and 20 minutes was allowed for stabilization. During this time arterial blood pressure, heart rate and respiration were carefully monitored to establish a steady state. Stabilization was assumed once these parameters remained constant for a period of 5 minutes. The observation period was only commenced once the halothane concentration was reduced to between 1 and 1.5 % and the animal was breathing diaphragmatically. Abdominal or erratic respiration was regarded as a sign of too deep a level of anaesthesia, and rapid ventilation, increasing blood pressure and heart rate indicated too light a level of anaesthesia.

1.3.14 Microsphere techniques

1.3.14.1 Introduction

The measurement of cerebral blood flow, organ blood flow and cardiac output was by a radiolabelled tracer microsphere technique. The method was based on the technique used in the animal laboratory at the Department of Physiology, University of Pennsylvania, Philadelphia. The technique employed by Professor Wagerle and his staff is in turn based on that published by Heymann (Heymann, 1977).

Different types of indicators injected into the intravascular compartment have for many years been used to study the circulation. Solid foreign particles have been employed in the measurement of organ blood flow, distribution of cardiac output, and the degree of shunting through various organs. The ability to label these particles with radionuclides, making them easily detectable or quantified, has been a major recent advance.

The nuclide-labelled microspheres used in this study were ion-exchange beads (styrene-divinyl benzene copolymer). They are insoluble, plastic, and have a specific gravity of about 1.3 as compared to 1.05 for whole blood. They are available in sizes ranging from 10 to 35 microns in diameter with a choice of 6 stock gamma emitter nuclides (New England Nuclear,Boston, USA). The nuclide is incorporated into the plastic and therefore the number of counts per minute detected are related to sphere volume. It is essential to note that flow rate measurements are made by the distribution of the microspheres, and that the radionuclide label only provides a means by which the number of microspheres can be accurately calculated. It is thus important that the size and shape distribution of the injected microspheres be constant. The quality checks recommended prior to microsphere usage are outlined below.

1.3.14.2 Microsphere preparation

1.3.14.2.1 Diameter/percentage abnormality

Checking of the diameter range and percentage abnormality of each batch of microspheres was carried out using the technique suggested by Heymann (Heymann, 1977). A microsphere batch will usually be rejected if the size range for 15 micron spheres is greater than 10 microns, if the average size is 5 microns greater or smaller than the size ordered, or if there are more than 1% damaged or abnormally shaped spheres.

1.3.14.2.2 Nuclide specificity

Nuclide specificity of each batch of microspheres was determined by observation of the output display of a suitably calibrated multichannel pulse height analyser. The spectrum of the batch was examined for extraneous peaks or other abnormal spectral manifestations.

1.3.14.2.3 Specific activity

Specific activity of each batch of microspheres was also determined. This was carried out by streak application of a drop of well vortexed microsphere solution onto a 1cm square piece of

mm graph paper. Five pieces of graph paper were prepared for each batch. These were examined under a light microscope at 60X magnification and the number of microspheres on each piece of paper counted 3 times. The graph paper was then placed flat on the bottom of a counting vial and the total number of counts for each piece of paper determined in the gamma counter. The average number of microspheres and the counts per minute for each piece of paper were obtained, and the counts per microsphere were calculated. The counts per microsphere of each piece of paper were averaged and the value used for that batch of microspheres. The 5 samples were then placed in a single counting vial and served as standards to be counted with each batch of tissue specimens.

1.3.14.2.4 Choice of microsphere radiolabels

1.3.14.2.4.1 Phase 2

The radiolabelled microspheres used in this study were cerium-141, strontium-85 and scandium-46. This combination of microspheres has been repeatedly used by Prof. L. Craig Wagerle (Wagerle,1987).

1.3.14.2.4.2 Phase 3

The radio-labelled microspheres used in this phase were as follows: Cobolt-57, Chromium-51, Tin-113, Ruthenium-103, Niobium-95, and Scandium-46. This combination of microspheres has been used previously (Hansen, 1984) and found to be a suitable combination with adequate spectral separation.

1.3.14.3 Mixing/suspension of the microspheres

The stock solutions of microspheres were shaken vigorously on a vortex mixer for between 2-4 minutes. Although sonication was recommended this was not possible in the phase 2 study but was carried out in the phase 3 experiments. Approximately 0.7 - 0.9 million microspheres were then drawn up into a tuberculin (1ml) syringe, and transferred into the specially prepared injector vial (Hernandez, 1980). The injector vial was then shaken for 20-30 seconds on the vortex mixer prior to injection.

1.3.14.4 Injection

The accurate measurement of blood flow by this technique is dependant on the uniform mixing of the microspheres at the site of injection and on their similar concentration in all arteries downstream from the site of injection. The two main factors that

will affect this uniformity of concentration are the site of injection and the number of microspheres injected (Buckberg, 1971). To obtain adequate mixing before the first major arterial branching, the microspheres should be injected as far as possible from that point. When measuring the distribution of systemic blood flow injection should be into the left atrium (Buckberg,1971), but injection into the left ventricle, as used in these studies, has been shown to allow accurate measurement (Buckberg, 1971; Hales,1974). For technical reasons it was decided to inject the microspheres via the left ventricular catheter in these studies.

The vial containing the microspheres was connected to the left ventricular catheter and the contents were injected by flushing 3 ml of warmed saline through the vial over a 20 second period. Continuous monitoring of arterial pressure and cardiac rhythm was undertaken during the injection period to note blood pressure changes or cardiac arrythmias. Special care was taken to prevent the inclusion of air emboli in the catheters during connection and disconnection of the tubing, and 0.9% saline was used to fill any spaces in the connectors.

1.3.14.5 Withdrawal of reference blood

For 10 seconds prior to, during, and 60 seconds after the injection of microspheres, a reference arterial blood sample was withdrawn from the left brachial artery. Withdrawal was at a

constant rate of 1.27 ml per minute using a Harvard withdrawal pump (Harvard Apparatus, Millis, MA). This process was carried out for each microsphere injection period and all reference blood samples for each animal were drawn up into a single syringe.

1.3.14.6 Transfer of samples to counting vials

At the completion of the experiment the syringe was removed from the withdrawal pump. The blood was then injected into counting vials which had previously been prepared with 0.5ml of distilled water in order to facilitate haemolysis and microsphere sedimentation. The glass syringe was flushed out with distilled water and the flush also placed in counting vials to ensure that all microspheres were counted. The vials were filled to a level of 2.5 cm to minimise geometric counting error.

1.3.14.7 Sample counting and processing

1.3.14.7.1 Gamma Counter

The gamma-counter used in these experiments was a Packard Autogamma Scintillation Spectrometer, Packard Instrument Co., Downers Grove, IL). A computer (M & PD, Mercedes Datacor) was used to correct for spillover counts from the different isotopes

and to calculate blood flow (Heymann, 1977). The computer program was written by Prof. L. Craig Wagerle and has been repeatedly validated.

The counting was carried out by the author in the Dept.of Nuclear Medicine at the Groote Schuur Hospital under the direction of Dr. J. Byrne Ph.D. and Mr. B. Norris.

1.3.14.7.2 Energy windows

1.3.14.7.2.1 Phase 2

The energy windows were set at the following:

Cerium-141:	125 keV - 170 keV
Strontium-85:	460 keV - 550 keV
Scandium-46:	820 keV - 1060 keV

1.3.14.7.2.2 Phase 3

The energy windows were set at the following:

Cobolt-57:	100 kev - 160 kev
Chromium-51:	250 kev - 320 kev
Tin-113:	321 kev - 400 kev
Ruthenium-103:	420 kev - 580 kev

Niobium-95: 590 kev - 780 kev

Scandium-46: 900 keV - 1300 keV

1.3.14.8 Data analysis and computation

Nuclide separation was performed using standard methods for differential spectrometry (Heymann,1977). Cerebral blood flow was calculated with the formula Q = (At X Qr).Ar-1.(Wt X 100), where:

Q = flow to tissue (ml.min-1.100g-1)

Qr = reference blood withdrawal rate

At = activity of the tissue

Ar = activity of the reference blood

Wt = tissue weight

Cardiac output (ml.kg-1.min-1) was calculated as:

(Ai X Qr).Ar-1.body wt-1

where Ai = total activity injected

Total activity was calculated from the volume of stock microsphere solution injected.

The calculations were made using a programme written by Professor L.Craig Wagerle and used in his laboratory for all microsphere studies. The formulae are all derived from the standard text on microsphere use in circulatory physiology (Heymann,1977).

1.3.15 Prevention of air embolism

The injection and removal of fluids via vascular catheters exposes the animal to the dangers of air embolism. Care was taken to ensure that air was not inadvertantly injected into either the arterial or the venous lines during specimen withdrawal. At times when disconnection of catheters was required, the exposed ends of the catheters were filled with normal saline from a syringe before reconnection. This prevented the introduction of air bubbles into the vasculature when the lines were flushed.

1.3.16 Method of laryngoscopy

Laryngoscopy was performed with the animal still partially anaesthetised. The animal's head was removed from the head-box and supported with the right hand. The tongue was displaced upwards, the mandible elevated and the neck extended.
To allow easier visualization of the epiglottis, pressure was applied to the anterior aspect of the neck with the little finger of the right hand. The blade of the laryngoscope was then

introduced into the mouth and used to elevate and deviate the tongue. During spraying in phase 2 the laryngoscope blade was not manipulated any more than was necessary to expose the glottic region. During intubation however, the tip of the blade was deliberately used to probe the posterior pharynx, elevate the epiglottis, and stimulate its superior and inferior aspects. This procedure was continued for a timed period of one minute. At the one minute signal endotracheal intubation was started.

1.3.17 Laryngeal spraying

1.3.17.1 Phase 2

Spraying of the larynx was carried out under vision in phase 2. The laryngoscope blade was used to elevate the tongue and mandible to expose the larynx and vocal cords. The laryngoscope stimulus was kept to a minimum and the larynx and cords were sprayed with 4 puffs of either saline or 2% lignocaine using a modified Forrester spray. This corresponded to a dosage of 8mg of lignocaine since the aerosol spray delivered 0.1ml of solution per spray.

1.3.17.2 Phase 3

The larynx was sprayed without laryngoscopic visualization in an effort to induce as little stimulation as possible.
The animal's head was removed from the head box and the time noted on the computer. One minute signals were then generated by the computer to allow accurate standardization of the procedure. The animal was kept in the supine position and its neck was extended. The tongue was held up to ensure adequate exposure of the pharynx.

In phase 3 a modified Forrester spray (Forrester, 1974) was used with a solution of 2% lignocaine. The distal end of the aerosol tube was gently introduced into the mouth and the palate and tongue were sprayed (1 puff). The aerosol spray tip was then pushed further into the mouth and eventually into the throat. Three puffs were administered as the tip was withdrawn. The animal's head was then replaced in the head-box.

Aerosolized solutions of lignocaine have been used to effect anaesthesia of the mucosa (Abou-Madi et al ,1975; Laurito et al, 1988). This method allows topical anaesthesia without the effects of mechanical stimulation (laryngoscopy or mucosal irritation). Efficient delivery of an aerosolized solution was impossible in the anaesthetised piglets without inducing some degree of mechanical stimulation, and for this reason it was decided to use the described method. Bromage and Robson (1961) reported that

blood levels of lignocaine after endotracheal administration were extemely variable. Peak blood levels in their study occurred at any time ranging from 5 minutes up to 25 minutes after administration. They recommended that the dosage should be restricted to a maximum of 6mg/kg in an effort to prevent levels from rising above 10 micrograms/ml. Adriani (1956) has asserted that local anaesthetic drugs are absorbed very rapidly through the alveoli, and that the resuting blood levels rise almost as fast as by intravenous injection.

In the present study, despite the desirability of serum lignocaine levels these measurements were not possible, due to financial and manpower constraints. There is conclusive evidence in adults that lignocaine is rapidly (albeit erratically) absorbed from the laryngotracheal region (Adriani, 1956: Bromage and Robson, 1961; Viegas and Stoelting, 1975; Rosenberg et al, 1980), but despite this there is very little published data on absorption rates in neonates. The fact that the drug may have been variably absorbed must be borne in mind when assessing the results.

1.3.18 Endotracheal intubation

Endotracheal intubation was initiated following 1 minute of laryngeal and pharyngeal irritation as described above. A 3mm shouldered Portex clear-plastic endotracheal tube was used in all cases. Under direct vision the tip of the tube was used to probe

the vocal cords for a period of 20 seconds. The tube was then introduced between the vocal cords and advanced a distance of approximately 1cm. The laryngoscope was then withdrawn and the tube held in place for a period of 2 minutes. The animal was then extubated and the head-box replaced.

1.3.19 Removal from the operating table

1.3.19.1 Histology study

At the end of the phase 1 experiment (approximately 30 minutes from the time of intubation) all catheters, except one umbilical vessel catheter, were removed. The incisions were closed with a continuous silk suture and dressed. The capped umbilical vessel catheter was left protruding. Following removal of the bolt the cranial wound was closed in two layers with silk.
At the end of the procedure the halothane was switched off and the animal was removed from the table and weighed.

Post operatively the phase 1 animals were placed in a comfortable warmed area and allowed to awake. Initially they were kept separate but it was found that they became distressed when alone. When all animals were kept together it appeared that close bodily contact had a calming effect and the animals remained undistressed.

The animals remained in the recovery area for a period of 3 - 4 hours and were then sacrificed by the injection of 5ml of a 20% solution of thiopentone sodium (Intraval, May and Baker) into the umbilical vessel catheter. Death was instantaneous.

1.3.19.2 Microsphere studies

At the end of the experiment the animals were sacrificed by injection of 5ml of a 20% solution of thiopentone sodium (Intraval, May and Baker) into the left vetricular catheter. The animals were then removed from the table and weighed. The left ventricular catheter was left in-situ to allow confirmation of its position at dissection.

1.3.20 Specimen storage

The animals were decapitated following sacrifice, and the brains were removed and placed into bottles with 10% formalin-saline.
In the phase 2 microsphere study the carcasses were dissected and the heart, lungs, kidneys and adrenal glands, liver and spleen were removed and placed in 10% formalin-saline. In addition the masseter muscles were dissected out in both phase 2 and 3 and stored with the other organs. In the phase 1 brain histology studies the carcasses were incinerated.

The brains remained in the 10% formalin-saline until such time as they were suitably firm to allow cutting. This time period was usually between 2 and 3 weeks.

1.3.21 Preparation of histology sections

1.3.21.1 Brain sections

Serial coronal slices of the brain were then prepared with between 12 and 14 brain slices being taken from each brain.

1.3.21.2 Tissue preparation

Routine haemotoxylin and eosin staining techniques were used in all sections, and in certain cases melanin and specific neural tissue stains were used. All slide preparation was carried out by Mrs. Anne Smith of the Dept. of Neuropathology at the University of Cape Town Medical School.

1.3.21.3 Staining and mounting

The slides were examined independantly by two members of the neuropathology department, Dr. R. Bowen and Mr. C. Gouveia, who were unaware of the study group from which the specimens had come.

1.3.22 Preparation of specimens

1.3.22.1 Regional brain dissection

1.3.22.1.1 Specific areas identified

1.3.22.1.1.1 Preparation

The dissection of the brain in the microsphere studies was much more complex than in the histology studies where brain slices were required. For regional brain blood flow studies the areas in question had to be dissected out as accurately as possible. To achieve as representative a region as possible the brain was again sliced into coronal sections. The slices were positioned at the optic chiasma, at the level of the Trigeminal nerves, at the junction of pons and medulla and at the juction of the medulla and upper cervical spinal cord. By dividing the brain up in this

way it was possible to identify the separate areas and dissect them out. The dissection of all of the brain specimens was carried out by the author.

1.3.22.1.1.2 Cerebral grey matter

Cerebral grey matter was collected by removing the peripheral layer of tissue from each coronal section. In areas were it was difficult to distinguish white and grey matter only that definitely seen to be grey was dissected out. Right and left specimens were taken for comparison.

1.3.22.1.1.3 Cerebral white matter

White matter was dissected from the coronal sections in the same way as described above. Generally only the large fibrous collections were taken with care being excercised to avoid grey matter. Right and left specimens were taken for comparison.

1.3.22.1.1.4 Cerebellum

The cerebellum was removed in its entirety by severing the cerebellar peduncles. The whole cerebellum was then dissected and placed in counting vials as a single tissue.

1.3.22.1.1.5 Caudate nucleus

Caudate nucleus tissue was identified in the floor of the anterior horn, and the tissue specimen removed consisted mainly of the head. This was dissected free from the corpus callosum superiorly, and separated from the lentiform nucleus by division along the anterior limb of the internal capsule. No attempt was made to dissect out the body or the tail of the caudate nucleus.

1.3.22.1.1.6 Thalamus

All three thalamic nuclei (medial, anterior, and lateral) were grouped together as a single specimen and no attempt was made to separate the white matter of the internal medullary lamina. The majority of the thalamic tissue specimen consisted of the pulvinar. Care was taken to remove only thalamic tissue between the medial boundary of the 3rd ventricle, and the lateral boundary of the internal capsule.

1.3.22.1.1.7 Hippocampus

The hippocampus specimen was "shelled out" in its entirety along the length of the floor of the inferior horn of the lateral ventricle. The anterior (pes hippocampi), and the posterior connections of the hippocampus were severed to facilitate removal of the specimen en masse. The white fibres of the alveus were not dissected off the major grey matter bulk of the organ.

1.3.22.1.1.8 Midbrain

The midbrain specimen was regarded as that piece of tissue extending from the optic chiasm cranially, to the oculomotor nerve caudally, and consisted of the corpora quadrigemina and the cerebellar peduncles.

1.3.22.1.1.9 Pons

The pons specimen was standardised as that piece of tissue extending from the oculomotor nerve cranially, to the abducens nerve caudally and included the root of the trigeminal nerve.

1.3.22.1.1.10 Medulla

The medulla specimen extended from the abducens nerve cranially to the first pair of cervical nerves.

1.3.22.1.1.11 Upper cervical

The upper cervical specimen extended from the first cervical nerve caudally, and usually included a 1 - 1.5 cm length of upper cervical spinal cord.

1.3.22.1.2 Tissue handling

The pieces of brain tissue were placed on filter paper to remove excess formalin and then transferred to the counting vials. Tissue was packed to a height of approximately 2.5cm in each vial to minimise geometric counting error. The inside of each vial was cleaned above the 2.5cm level and the vial top was replaced.

1.3.22.1.3 Weighing of specimens

The animals were weighed using an Avery laboratory scale.

All specimens for microsphere studies were weighed to an accuracy of 4 decimal places of a gram using an electronic Metzler mass. The counting vials were weighed prior to tissue dissection, and then again once the specimen had been placed inside. True wet fixated tissue weight was taken as the difference between these two values.

1.3.22.2 Organ dissection

1.3.22.2.1 Introduction

The other organs were prepared in much the same way to the brain. The organ was removed from the preservative, dried on a piece of filter paper, and dissected into small cubes of tissue. A representative piece of tissue was removed in the case of an organ too large to be placed into two vials, and in each case this piece of tissue was removed in exactly the same way. The dimensions of the representative sections are described below.

1.3.22.2.2 Masseter muscle

The masseter muscle once dissected, was, in all cases, small enough to be placed in a single counting vial.

1.3.22.2.3 Kidney

The kidney was filleted and the pelvi-ureteric junction was removed. The remaining piece of kidney was cut into a cube 1cm X 1cm and placed into the vial.

1.3.22.2.4 Adrenal gland

In all cases the adrenal glands were small enough to fit into single vials.

1.3.22.2.5 Liver

The representative piece of liver was taken from the area of liver beneath the gall bladder in all cases. A 1cm X 1cm piece of liver tissue was dissected and placed into the vial.

1.3.22.2.6 Spleen

In all cases the entire spleen was dissected and placed in a single counting vial.

1.3.22.2.7 Heart

In all cases the piece of heart tissue was removed from the left ventricular wall at the apex of the heart and included full wall thickness sections.

1.3.22.2.8 Lung

Lung tissue specimens (1cm X 1cm) were removed from the lingula (left) and the right middle lobe in each case.

2 Statistical methods.

2.1 Phase 1

The statistical analysis in the phase 1 study was by a 2-way analysis of variance (ANOVA). Individual comparisons were made using the method of least significant differences. Homogeneity of variances was tested by means of Bartlett Criteria (Snedecor and Cochran, 1980).
The Fishers Exact Test was employed in frequency count analysis where expected cell values were less than 5 (Siegel, 1956).

2.2 Phase 2

The data in phase 2 was analysed using an analysis of variance where the logarithm of the ratio of the values at baseline, post-laryngoscopy/spray and post-laryngoscopy/intubation were used. The componants in the analysis were:

-Treatment groups (Saline vs Lignocaine)
-animals within treatment groups
-stages
-treatment X stage interaction
-error

2.3 Phase 3

An analysis of variance was carried out, as in phase 2, using the components mentioned below. Since both treatment groups in the phase 3 experiment were already differently treated at the baseline estimation the baseline values could not be corrected for individual variation. Components:

-treatment groups (Control vs Lignocaine)
-animals within treatment groups
-stages or time periods (6 timepoints)
-treatment X stage interaction
-error

In those instances where large coefficients of variance were noted (indicative of skewed data) the logarithms were used. In the blood flow and resistance data analysis the geometric means were plotted along with their 95% confidence intervals. The confidence intervals were computed using the means of the log. data, and then back-transformed. This led to asymmetric confidence intervals in some cases.

All analyses were carried out by Dr. D. Van Schalkwyk of the Medical Research Council Institute of Biostatistics. The SAS statistical computer package was used. Differences between treatments and amongst timepoints were tested using the Neuman-Keuls Studentized range method for multiple comparisons. In those cases where multiple comparison tests were not performed on treatment X Stage combinations the t-tests were carried out but the level of significance was taken at $p < 0.01$ rather than $p < 0.05$.

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1 Sample groups - Phase 1

Seventeen animals were evaluated. One animal was excluded when it died suddenly soon after induction of anaesthesia. The cause of death appeared to be malignant hyperthermia. Since this animal had not undergone any experimental intervention, it was removed from the study. The Control group thus consisted of 6 animals and the Intubation group of 11 animals.

2 Table design

The following tables have been set out in most cases as ANOVA (analysis of variance) tables. Unless specifically indicated all analyses have been by one way analysis of variance. Data has been presented as the mean +/- standard deviation. The least significant difference for the concerned parameter is quoted at the bottom of each table. Unless otherwise stated the least significant difference value corresponds to a p value of 0.05.

3 Monitored parameters - Phase 1

3.1 Blood pressure

3.1.1 Mean arterial pressure

Mean arterial pressure (MAP) changes are presented in Table P1-1. In the 6 Control animals, there was a small increase in the MAP from 50 +/- 5 mmHg to 53 +/- 11 mmHg ($p>0.05$), possibly related to the slightly increased PaCO2 level and the reduced pH.

In the 11 animals that were intubated the MAP rose acutely from 47 +/ 8 mmHg to a peak level of 68 +/- 12 mmHg ($p<0.01$) following the laryngoscopy/intubation.

TABLE P1-1: Mean arterial blood pressure changes following laryngoscopy/intubation in the Control group and the Intubation group.*

	BASELINE	PEAK	p
CONTROL GROUP (mmHg) (n = 6)	50 +/- 5	53 +/- 11	NS
INTUBATION GROUP (mmHg) (n = 11)	47 +/- 8	68 +/- 12 #	<0.01

*Data presented as: Mean +/- standard deviation

NS = Not significantly different from the baseline value

#Significantly different from the Control group ($p<0.01$)

Least significant difference = 10.0 ($p<0.05$)

= 13.5 ($p<0.01$)

3.1.2 Time-to-peak/time-to-baseline

Table P1-2 presents the time interval data.

TABLE P1-2: Time-to-peak and time-to-baseline for mean arterial pressure and heart rate following laryngoscopy/intubation.

	INTUBATION GROUP (n = 8)	
	MEAN ARTERIAL PRESSURE	HEART RATE
TIME TO PEAK (minutes)	4.1 +/- 1.6	2.2 +/- 0.7
TIME TO BASELINE (minutes)	13.8 +/- 4.9	6.0 +/- 4.0

*Data presented as: Mean +/- standard deviation

In the phase 1 study the time intervals were not completely documented because of equipment failure in the initial stages of the study. Data is only available for 8 of the 11 animals studied (Table P1-2) in the Intubation group. In the remaining 3 animals only the baseline and peak values were recorded.
The time-to-baseline value represents the time required for the parameter concerned to return to within one standard deviation of the the pre-stimulus level.

3.2 Heart rate

The heart rate changed minimally in the Control group (Table P1-3). In the Intubation group there was a larger increase in heart rate over the laryngoscopy/intubation period, but because of the large variances this increase was not significant. The time intervals are tabulated in Table P1-2.

TABLE P1-3: Heart rate changes following laryngoscopy/intubation in the Control group and Intubation group.*

	BASELINE (beats per minute)	PEAK (beats per minute)	p
CONTROL GROUP (n = 6)	146 +/- 12	148 +/- 14	NS
INTUBATION GROUP (n = 11)	146 +/- 23	163 +/- 24	NS

*Data presented as: Mean +/- standard deviation

NS = No significant difference from the baseline value

Least significant difference = 21.36 ($p<0.05$)

= 28.76 ($p<0.01$)

4 Blood gases

4.1 pH and base excess

Table P1-4 shows the blood gas results for the phase 1 study. The acid-base values changed minimally ($p>0.5$) in both of the groups. The pH values are within the normal range for neonatal piglets of this age.

TABLE P1-4: Phase 1 blood gas parameters at baseline, and following laryngoscopy/intubation, for the Control and Intubation groups.*

	CONTROL GROUP (n = 6)	INTUBATION GROUP (n = 11)	p
BASELINE pH	7.33 +/- 0.06	7.27 +/- 0.06	NS
POST EVENT pH	7.31 +/- 0.04	7.28 +/- 0.09	NS

BASELINE O2	243 +/- 80	285 +/- 76	NS
POST EVENT O2	252 +/- 149	236 +/- 114	NS
BASELINE CO2	57 +/- 7	63 +/- 9	NS
POST EVENT CO2	58 +/- 11	66 +/- 21	NS
BASELINE BE	0.5 +/- 3	-5 +/- 6	p<0.05
POST EVENT BE	-0.6 +/- 2	-7 +-/ 6	p<0.05

*Data presented as: Mean +/- standard deviation

#Value significantly different from baseline

NS = No significant difference between the groups (p = 0.05)

BE = Base Excess

Least significant difference for pH = 0.07 (p< 0.05)

Least significant difference for O2 = 109 (p< 0.05)

Least significant difference for CO2 = 15 (p< 0.05)

Least significant difference for BE = 5 (p< 0.05)

The Intubation group had insignificantly lower baseline pH and slightly higher PaCO2 values than those seen in the Control group. This may possibly be related to the slightly longer preparation time (approximately 5 minutes) required in the Intubation group due to the insertion of more brain tissue pressure monitors in this group of animals. Since these differences were not significant however they are difficult to interpret and do not necessarily represent clinically significant changes.

The mean pH and base excess for neonatal piglets has been reported as being 7.224 (BE = -3.9) at birth and increasing to 7.389 (BE = 0.5) by 24 hrs of age (Glawischnig and Schlerka, 1980). In piglets 1-3 days old the mean values are reported to range from 7.340 to 7.423 (BE = 0.4) (Kutas and Szabo, 1971; Andren, 1980).

4.2 PaCO2

The mean PaCO2 changed minimally in the Control group (57 +/-7 mmHg to 58 +/- 11 mm Hg). In the Intubation group however there was a slightly greater but still insignificant increase possibly related to airway obstruction during the procedure. The PaCo2

levels were indicative of hypercapnia in piglets of this age. Glawischnig and Schlerka (1980) reported normal PaCO2 levels for neonatal piglets to range from 61.8 mmHg at birth to 43.6 mmHg at 24 hrs. The hypercapnia seen in this study was most likely related to CO2 rebreathing - despite the soda-lime canister. This was further compounded by a degree of respiratory depression associated with the inhalational anaesthetic. From these reference values it is obvious that both groups of animals were mildly acidotic on a respiratory basis, but because of the particular requirements of this study, the hypercapnia was accepted as an inevitable side effect of the chosen anaesthetic method. There was no significant difference in the PaCO2 values in or between either group at any time during the experiment.

4.3 PaO2

In all cases the animals were hyperoxic throughout the experiment. In no animal was the PaO2 less than 100 mmHg at any of the blood gas estimations. This was regarded as being important since it was intended that hypoxic cerebral blood flow changes were to be avoided. Normal PaO2 values are reported as ranging from 24 mmHg at birth (Glawischnig and Schlerka, 1980) to 63.8 mmHg at 2 days (Andren, 1982). The cerebral vasoconstrictor effects of a high PaO2 tension are discussed elsewhere in this text.

5 Brain interstitial tissue pressure

Table P1-5 shows the brain interstitial tissue presure changes following the stimulus.

TABLE P1-5: Phase 1 brain interstitial tissue pressure changes following laryngoscopy/intubation.*

	CONTROL GROUP (n = 2)	INTUBATION GROUP (n = 9)	p
BASELINE (mmHg)	0	0	
PEAK (mmHg)	-2.5 +/- 0.5	7.5 +/- 8.4	#0.14

*Data presented as Mean +/- standard deviation

No significant difference between the two groups

The trend in the brain tissue pressure (Table P1-5) was towards a reduction in the control group (-2.5 +/- 0.5 mmHg) and an increase in the Intubation group (7.5 +/- 8.4 mmHg). This increase was maintained for a mean duration of 2.3 +/- 1.1 minutes. Unfortunately the variances are too large and the sample sizes too small, to allow any meaningful interpretation, other than that the brain interstitial tissue pressure tends to increase during laryngoscopy/intubation.

6 Histology

Table P1-6 shows the macroscopic and microscopic findings of the histologic studies. The table includes an indication of the brain section (in brackets) in which the pathology was found. The sections were numbered from rostral to caudal, and as described in the methods each slice was approximately 1cm in width. Obviously it was not possible to ensure that each numbered section corresponded exactly to all of the other sections of the same number, but as far as possible the selected sections are representative of the same brain regions in the different animals.

The pathologist who reviewed the histology was aware that in some of the animals an intraparenchymal catheter had been placed, and knew the approximate area of catheter insertion. However at the time of examining the slides he was not told from which group

each slide had derived. Although it was not possible to differentiate on purely morphological grounds between catheter induced bleeding and "real" haemorrhage, a histological assessment as to whether the observed bleeds were likely to be traumatic or "real" was recorded in each case. Any bleeding in the area of catheter insertion that was considered to be iatrogenic (usually within sections 2 to 4) was not included in the analysis.

It is important to note that in none of the reviewed slides was there any evidence of iatrogenic haemorrhage in the basal areas, and in addition, in those slides in which iatrogenic bleeds were suspected, these bleeds were always focal subarachnoid bleeds.

Although the brain was sliced into 12 coronal sections for the slide preparation, there were obviously areas that were not visualised microscopically. However, in those brain sections that were examined there was no histologic indication of any communication between the catheter insertion zone and the ventricular system. Further proof that the observed haemorrhages were more likely to have resulted from the laryngoscopy/intubation than from the insertion of the brain tissue pressure catheter was provided by the fact that no intraventricular haemorrhages were noted in either of the 2 control animals that had the catheter placed. In addition, of the 9 animals in the Intubation group that had catheters sited, there were none that had any evidence of lateral ventricle bleeds

in relation to the catheter. In those animals that demonstrated intraventricular haemorrhages, there were none that had bleeds higher than the 4th ventricle.

The interpretation of these slides was complicated by unavoidable subjectivity since there is a distinct paucity of reference material concerning histology in the neonatal piglet brain. The significance of the small amounts of blood and proteinaceous material seen in the central canal area of the Intubation group is also difficult to assess, but since these changes were not seen in the control animals it is likely that the laryngoscopy/intubation was in some way responsible.

Table P1-6: Histopathologic findings in the Control (C) and Intubation (I) groups. The section number is in brackets.

No.	SEX	GROUP	BTP	HISTOLOGY (SECTION)
1	M	C	*	NO ABNORMAL FINDINGS
2	F	C	*	FOCAL SUBARACHNOID BLEED (3,4) FOCAL INTRAPARENCHYMAL BLEED (4)
3	M	C		NO ABNORMAL FINDINGS
4	F	C		NO ABNORMAL FINDINGS
5	F	C		NO ABNORMAL FINDINGS
6	M	C		NO ABNORMAL FINDINGS

7	M	I		SUBEPENDYMAL CONGESTION (1)
8	F	I		MENINGEAL CONGESTION (1)
				SUBARACHNOID BLEED (3)
				CENTRAL CANAL BLEED (6)
9	M	I	*	4TH VENTRICLE BLEED (6)
				CENTRAL CANAL BLEED (7)
10	F	I	*	INTRAPARENCHYMAL BLEED (4)
				CENTRAL CANAL BLEED (6)
11	M	I	*	4TH VENTRICLE BLEED (6)
				CENTRAL CANAL BLEED (7)
12	M	I	*	CENTRAL CANAL BLEED (7)
				CENTRAL CANAL PROTEIN (7)
13	M	I	*	FOCAL SUBARACHNOID BLEED (1)
				CENTRAL CANAL PROTEIN (6)
14	F	I	*	FOCAL SUBARACHNOID BLEED (3)
				CENTRAL CANAL BLEED (5)
				CENTRAL CANAL PROTEIN (5)
				CHOROID PLEXUS BLEED (6)
				HAEMOSIDERIN IN CHOROID PLEXUS (6)
15	F	I	*	SUBARACHNOID BLEED (1,2,6)
				CENTRAL CANAL BLEED (6)
16	M	I	*	SUBARACHNOID BLEED (5,6)
				4TH VENTRICLE BLEED (7)
				CENTRAL CANAL BLEED (5)
				CENTRAL CANAL PROTEIN (5,8)

17	F	I	*	SUBARACHNOID BLEED (2,3,4,6) INTRA VENTRICULAR BLEED (3,4) CENTRAL CANAL BLEED (6,8) FOCAL CELLULAR OEDEMA (4)

BTP = Brain interstitial tissue pressure catheter

* = BTP catheter inserted

In most cases of subarachnoid bleeding the neuropathologist could not be absolutely sure as to whether the bleeding was iatrogenic or whether it was true pathologic haemorrhage, and the bleeds were reported as they were seen, without any attempt to classify them. Note however that the majority of the central canal and intraventricular bleeds were found in sections 4 to 8, corresponding to the 4th ventricle and the more distal brain stem regions. In these central canal bleeds it was less likely that iatrogenic trauma played a role, both because of the distance from the parietal lobe, and due to the central nature of the pathology. In piglet 14 there was a definite choroid plexus bleed noted, with haemosiderin being clearly identified. In piglet 17 there was an area of cerebral oedema noted, and in this animal there was also evidence of an intraventricular bleed.

Plate 1 shows an example of a central canal haemorrhage. There is no obvious interruption of the ependymal lining seen. In no case in which central canal bleeds were noted was there evidence of intraventricular haemorrhage at any level higher than the 4th

ventricle. It is thus assumed that the blood seen in the central canal has originated from an area distal to the 4th ventricle in the basal region of the brain and is not blood that has tracked distally from a bleed higher in the ventricular system.

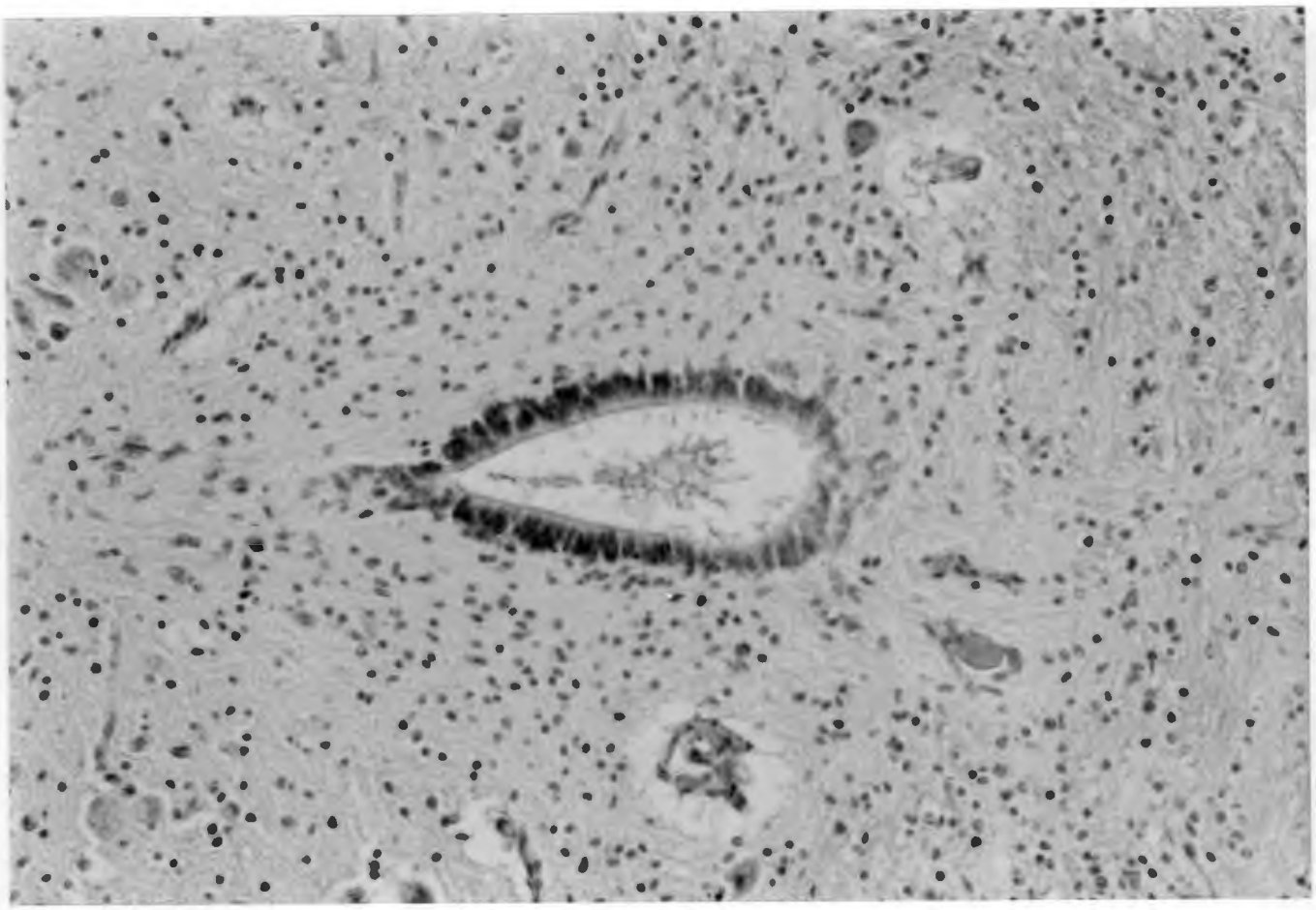

Plate 1.

Plate 2 shows a subarachnoid bleed.

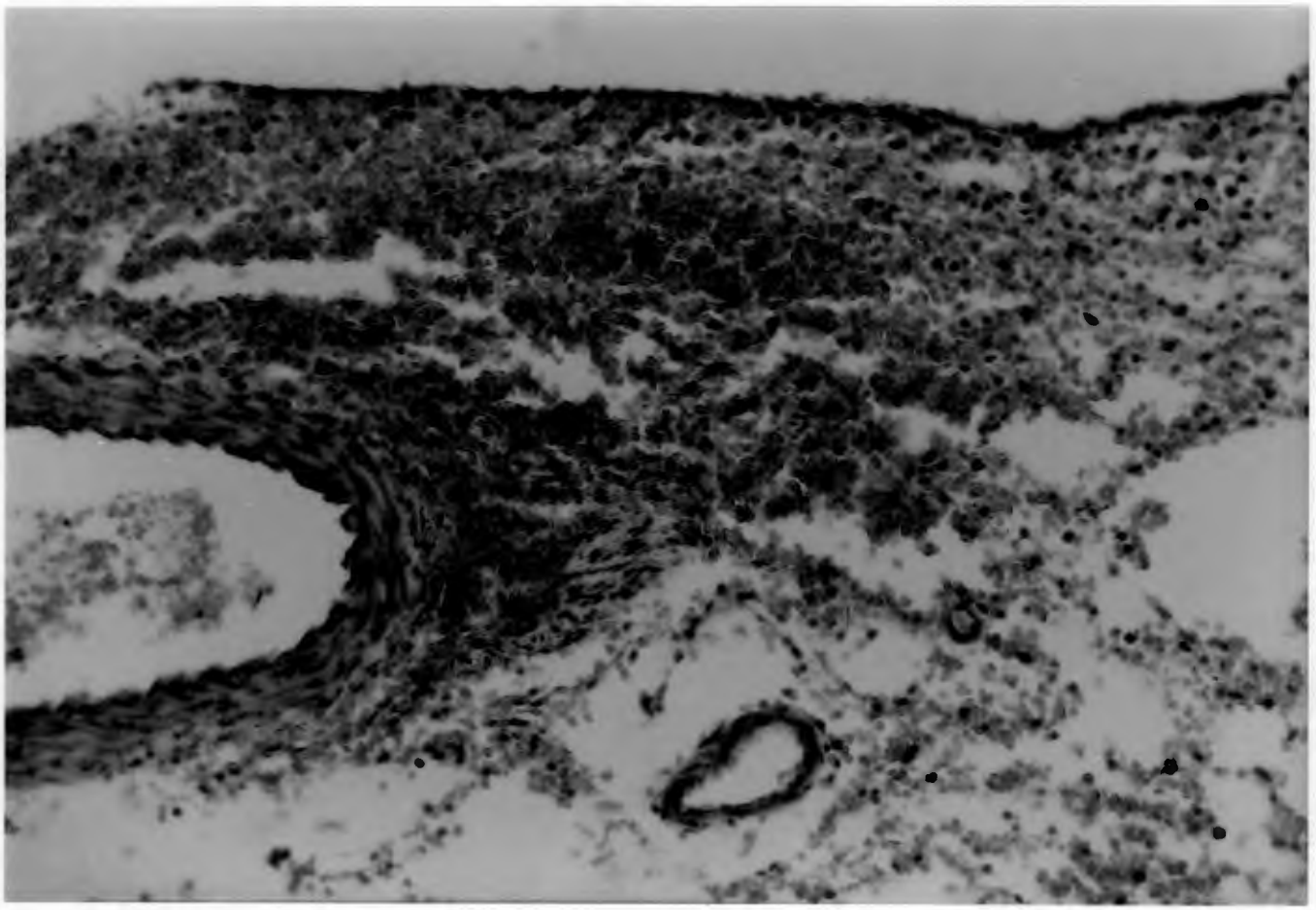

Plate 2.

In Plate 3 there is an example of an intraparenchymal haemorrhage.

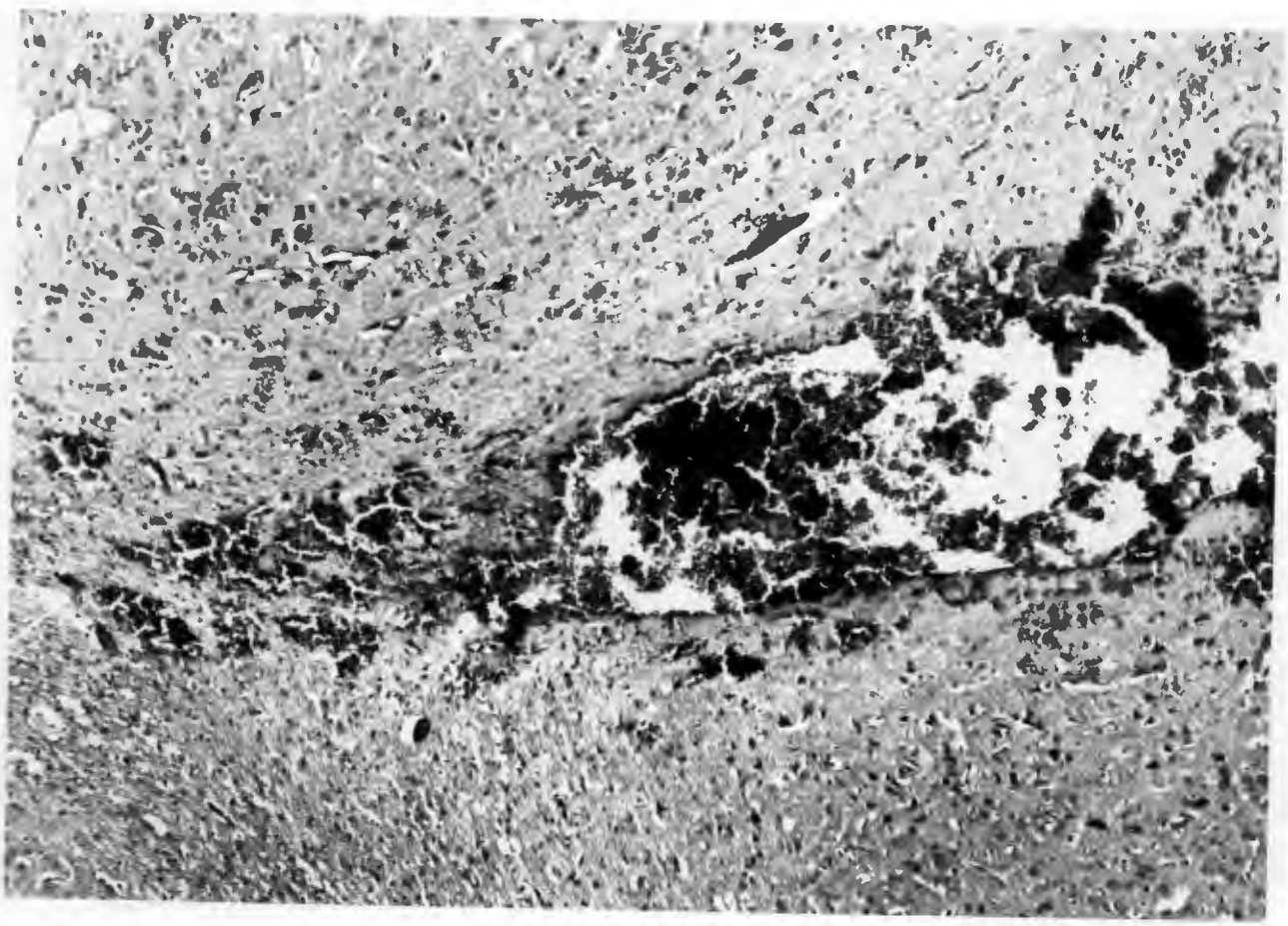

Plate 3.

Plate 4 shows central canal proteinaceous material, which is to be distinguished from obvious blood cells, and may be indicative of altered blood or protein extravasation.

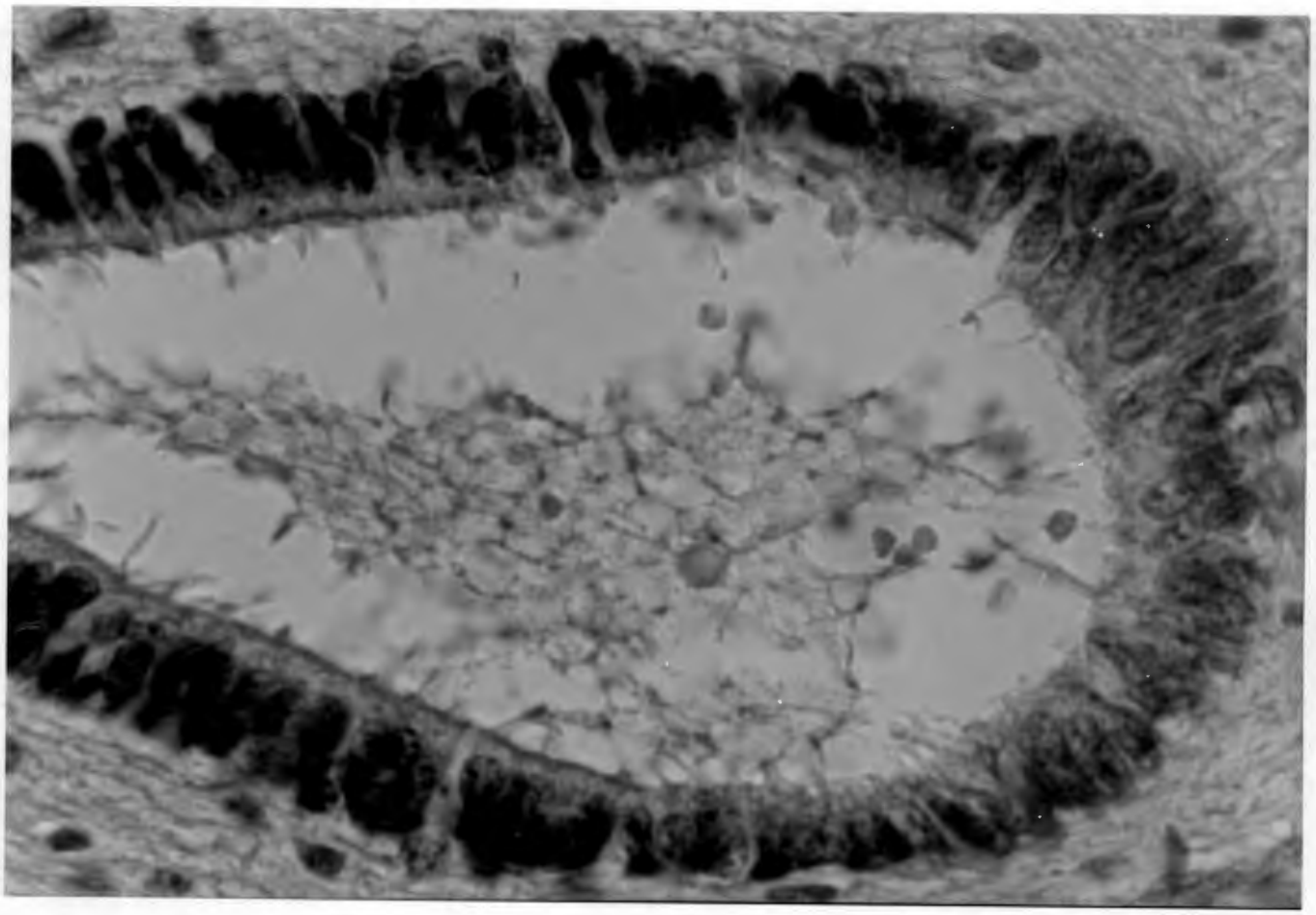

Plate 4.

Plate 5 shows an area of oedema.

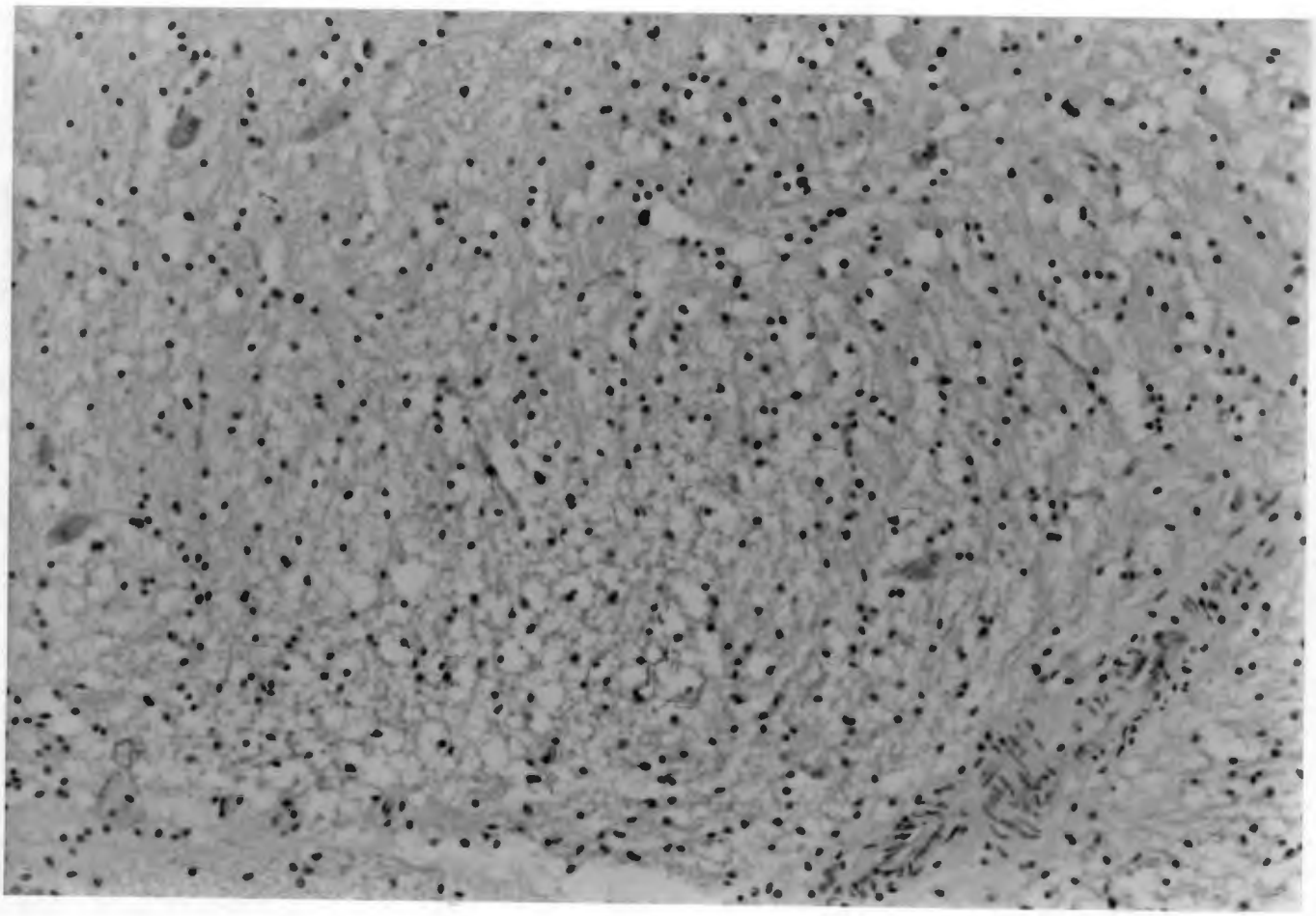

Plate 5.

Plate 6 shows a choroid plexus bleed. Note the haemosiderin granules which are considered as definite evidence of abnormal bleeding in this area.

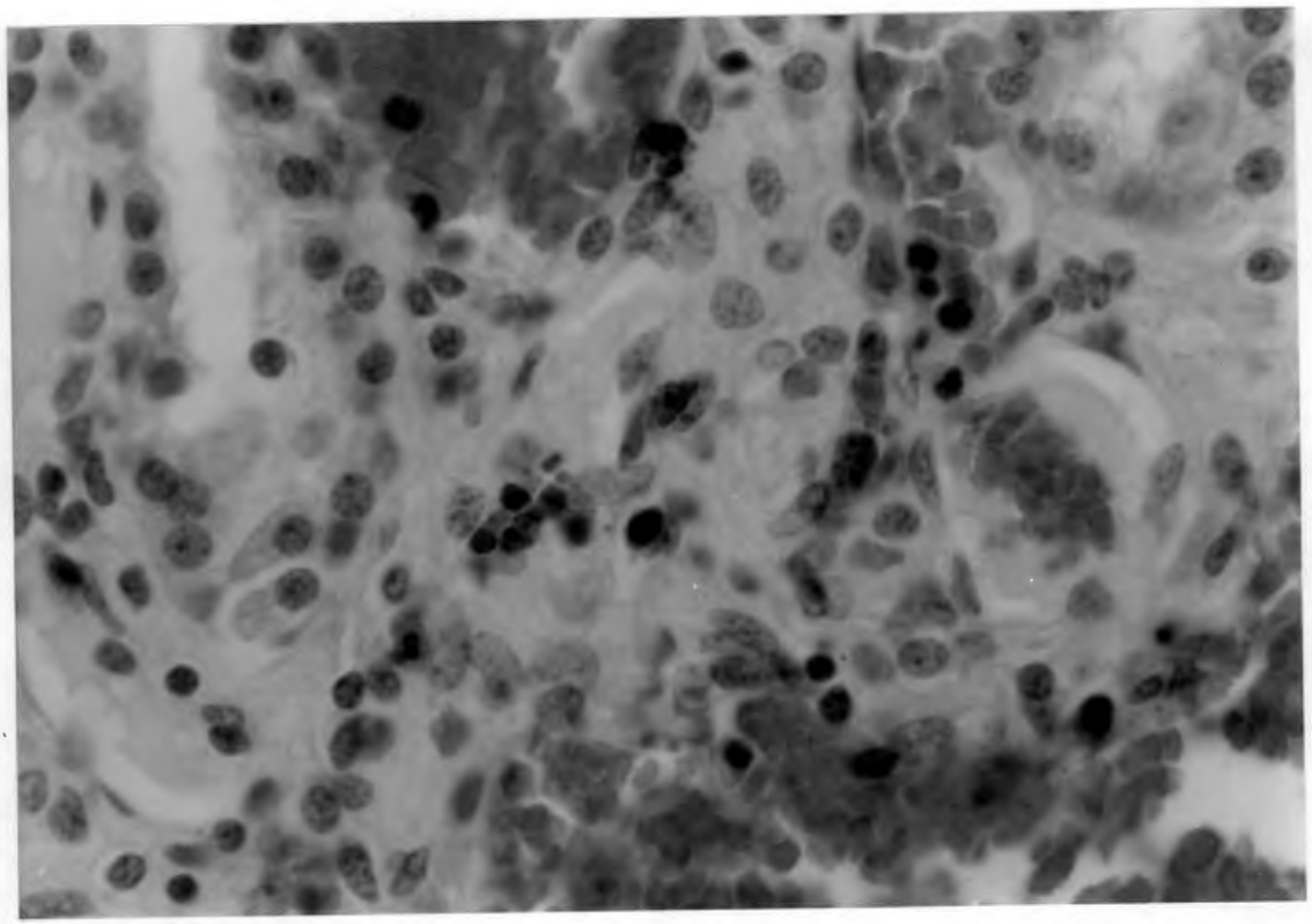

Plate 6.

Plate 7 shows an intraventricular haemorrhage.

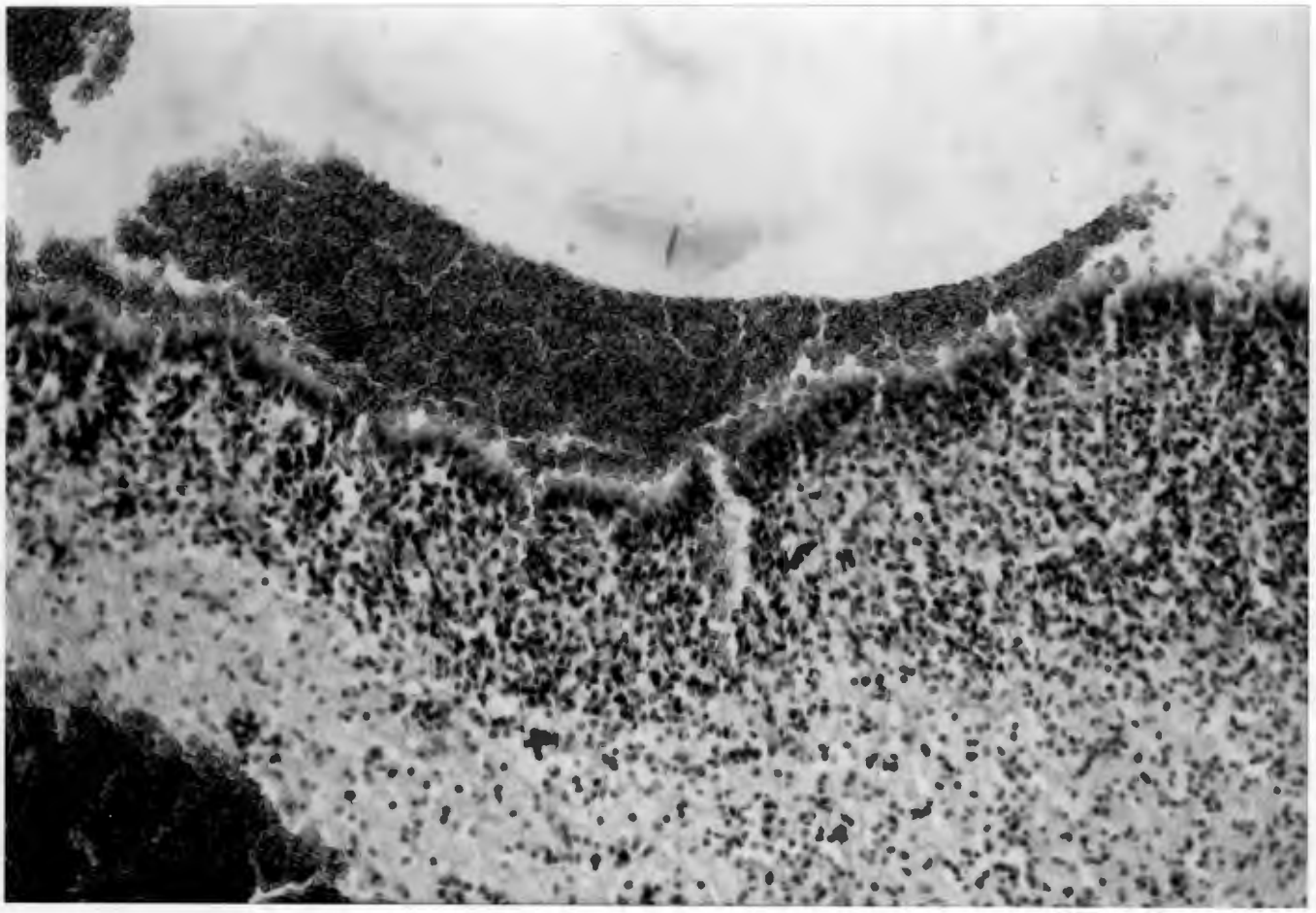

Plate 7.

There was a single animal in the Control group that showed evidence of haemorrhage. This was the animal described earlier that had a period of struggling and hypertension during the insertion of the second umbilical artery catheter. A peak MAP of 66 mmHg was attained that lasted approximately 2 minutes, and was associated with struggling and signs of distress. The animal settled well once stimulation was stopped and anaesthesia was deepened.

Analysis of the data using the Fisher's exact test showed that when the Control and Intubation groups were compared for total number of bleeds, there were significantly more bleeds ($p = 0.005$) in the intubated animals. Further calculation demonstrated that if only the bleeds in sections 6 and 7 were analysed, there was a highly significant difference between the two groups ($p = 0.002$), with the intubated animals showing significantly more bleeds.

7 DISCUSSION: Phase 1

Histologic evaluation of the acute effects of endotracheal intubation in neonatal animals less than 12 hours old has not previously been reported. The preliminary results of this study indicate that laryngoscopy and intubation in hyperoxic/ hypercapnic neonatal piglets may cause non-specific lesions of the brain stem with haemorrhage and protein extravasation in the 4th ventricle and the central canal. This seems an unusual distribution for cerebral haemorrhage resultant from hypertension, since most reports have implicated the periventricular cerebrum or the choroid plexus and lateral ventricles as the areas of bleeding (McDonald et al, 1984; Hambleton and Wigglesworth, 1976; Haggendal and Johansson, 1972; Lou et al, 1979; Goddard et al, 1980). One must remember that in most studies of hypertension-induced haemorrhage in experimental

animals, the beagle puppy, or other preterm models have been used. In these animals there is still a considerable amount of germinal matrix present, into which bleeding will readily occur (Takeshima and Tanaka, 1978; Goddard et al, 1980). The piglet model however, is significantly more neurodevelopmentally mature at term than the beagle puppy, and there is very little germinal matrix still in evidence (Oh, 1988). There may well be a different distribution of bleeding induced by moderate hypertension in the more mature neonatal brain. Intra ventricular haemorrhage is thought to be caused by rupture of the fragile capillary network located in the germinal matrix over the head of the caudate nucleus (Hambleton and Wigglesworth, 1976). In 10% of neonates, it is due to bleeding from the choroid plexus (Donat et al, 1978).

Mayhan et al (1986) reported on the disruption of the blood brain barrier in cerebrum and brain stem during acute hypertension (phenylephrine induced) in male Sprague-Dawley rats. These workers showed that the relative contribution of large and small vessels to total cerebral vascular resistance differed in the brain stem and cerebrum under control conditions, with a greater percentage of the resistance being accounted for by vessels < 60 micrometers in diameter in the brain stem. They also state that there appears to be a haemodynamic mechanism for greater protection of the blood brain barrier during acute hypertension in the brain stem. This mechanism is related to attenuation of increases in venous pressure and not to attenuation of increases

in arteriolar pressure. There is a greater pressure drop between arterioles and venules in the brain stem compared with the cerebrum during acute hypertension. This data suggests that small vessels and small vessel resistance play a major role in the regulation of the blood-brain barrier in the brain stem during acute arterial hypertension, and that the attenuation of increases in venous pressure may be the primary haemodynamic mechanism of protection in this area under these conditions. Why the major percentage of bleeds occurred in the brain stem in the present study is a matter for speculation. Whether the stimulation of the larynx and trachea results in a different pattern of vascular response, as compared to phenylephrine induced hypertension, must be considered. The intense stimulation of the larynx and trachea may cause the release of many vasoactive amines (see pages 48 -77), and in addition may recruit accessory neural pathways (page 51), possibly resulting in a very different blood flow pattern to that seen by Mayhan et al (1986). The effect of hypercarbia on the cerebral vessels is to disproportionately increase the blood flow (see pages 59 - 61) in the brain stem as compared to the cerebrum, and the possibility that this is due to an effect of the raised PaCO2 on the venous circulation exists, and warrants investigation. The combination of hypercarbia and a potent (and complex) sympathetic stimulus may account for the unexpected basal brain bleeds in the present study.

A larger proportion of cerebral blood flow is carried by the vertebral vessels in small animals (primates, dogs, cats and rabbits) as compared to human neonates (Schmidt, 1950). There is no data for piglets, but if one assumes that this is also the case in pigs, it may contribute to explaining the preponderance of lesions in the vertebrobasilar distribution in this study.

Sympathetic stimulation of the peripheral cerebral vessels (which are relatively better supplied with sympathetic innervation than the deeper arterioles (Edvinsson, 1975) may be responsible for shunting of blood into the vertebrobasilar distribution, where protective sympathetic vasoconstriction is less in evidence. Wagerle and Delivoria-Papadopoulos (1987) showed that under hypercapnic conditions, sympathetic stimulation caused pronounced CBF reduction in the cerebrum, hippocampus, and caudate nucleus (cerebral tissues), yet effects in the choroid plexus and masseter muscle were unaltered. Wagerle et al (1986) suggest that in newborn piglets sympathetic innervation is less dense in the hindbrain regions than in the forebrain regions. Sympathetic stimulation during hypercapnia may thus cause shunting of blood to those areas less well innervated, viz the hindbrain regions, resulting in excessive pressure pulses in the basal areas of the brain. As discussed above, this appears to sharply contrast observed effects of acute hypertension under hypercapneic conditions in adult animals, where the blood brain barrier damage was noted in the cerebrum, with sparing in the brainstem (Mayhan, 1986).

Cerebral blood flow in neonates is influenced by changes in systemic blood pressure (Lou et al, 1979), and most clinical and experimental data indicate that an abrupt increase in systemic blood pressure is important in the genesis of intraventricular haemorrhage (Goddard et al, 1980; McDonald et al, 1984).

Perlman and Volpe (1983) showed a prominent, consistent increase in blood flow velocity in the anterior cerebral arteries during endotracheal suctioning, which was associated with an increase in blood pressure and intracranial pressure. The mechanism by which the intracranial pressure is increased is very much a matter for speculation, but it is possible that the observed increase in PaCO2 (Table P1-4) might have contributed to the increase in BTP. Sympathetic stimulation leading to the release of catecholamines has beeen shown to result in hypertensive surges (Russel et al, 1981; Tomori and Widdicombe, 1969) and is attractive as a possible mechanism. This would imply that the raised blood pressure must overcome the protective sympathetic vasoconstriction of the cerebral vessels since sympathetic stimulation, without an increase in blood pressure, would result in a decrease in intracranial pressure of approximately 2-3 mmHg (Baumbach et al, 1983).

The cerebral bleeding in a control animal subjected to non laryngotracheal sympathetic stimulation severe enough to cause a hypertensive surge is interesting. Although this animal bled intraparenchymally, there was no evidence of central canal

bleeding. This supports the theory that laryngotracheal stimulation may contribute to a different pattern of bleeding to that of non laryngotracheal sympathetic stimulation.

Friesen et al (1987) speculate as to the contribution of venous stasis resulting from forced expiratory efforts and coughing that may be associated with awake or emergency intubation. This hypothesis appears to be supported by the observations of Perlman et al (1985) who showed wide fluctuations in cerebral blood flow velocity in preterm neonates with spontaneous ventilatory efforts out of synchrony with their mechanical ventilators. This is an unlikely explanation for the pathology noted in in the Intubation group in this study since there was no evidence of struggling or coughing during the laryngoscopy or intubation. In the single control animal which bled however, increased venous pressure resultant upon the struggling, may have played a role.

Venous stasis during laryngoscopy and intubation may be a cause of haemorrhage. Marshall et al (1984) showed that during laryngoscopy in 3 preterm neonates in whom nasal air flow was recorded, obstructive breaths were documented. Obstructed expiration during the procedure may result in elevated venous pressures and venous stasis. Pressure on neck veins and unnatural positioning of the head during laryngoscopy and intubation may possibly contribute to venous stasis and raised venous pressure, and accordingly, care was taken in this set of experiments to eliminate this as a contributory variable.

Stow et al (1988) studied the anterior fontanel pressure increase after laryngoscopy and intubation. They showed that anaesthesia reduced the increase in anterior fontanel pressure, and that this reduction was due to the decreased intrathoracic pressure changes noted when there was no coughing or straining. There was still however an increase in anterior fontanel pressure during the procedure due to an increase in mean arterial pressure. It has been shown that asphyxia may cause significantly increased cerebral blood flow in neonatal piglets (Laptook et al, 1982). Airway obstruction often seen with prolonged laryngoscopy and intubation may well cause a degree of asphyxia, but whether it is sufficient or prolonged enough to result in increased brain blood flow is not certain.

Lou and co-workers observed a passive systemic arterial pressure/ cerebral blood flow relationship after prolonged asphyxia in near-term fetal sheep (Lou et al, 1979). The animals in this study were not subjected to prolonged asphyxia, but did undergo prolonged hypercarbia. Hypercarbia has also been shown to interfere with cerebral autoregulation (Rapela and Greer, 1964) with the most pronounced effects occurring in the brain stem (Hansen et al, 1984). Loss of cerebral autoregulation combined with a disproportionate increase in regional cerebral blood flow induced by the combination of hypercarbia and sympathetic stimulation is hypothesised as the cause of the observed hindbrain bleeds in this study.

Brubakk (1987) has shown that prolonged hypercapnia results in spontaneous elevations of mean arterial blood pressure, and pressure passive increases in brain blood flow in neonatal piglets. These changes may be related to catecholamine release mediated through concurrent metabolic acidosis. It is unlikely that the rapid changes in blood pressure seen in this study were caused by hypercarbia, but superimposed on the background of hypercarbia-induced pressure passive cerebral blood flow, hypertensive surges resultant from the laryngoscopy and intubation may be transmitted into the cerebral circulation with injurious consequences.

The importance of cerebrovascular sympathetic regulation in neonates is still undefined, but in adults, sympathetic stimulation limits cerebrovascular vasodilatation, and reduces increases in CBF during hypercapnia, hypoxia and hypertensive episodes (Bill and Linder, 1976). The neonate, and in particular the stressed neonate, is often exposed to such conditions during delivery or resuscitation. Bearing in mind the four - fold less ground matrix in the neonatal blood-brain barrier capillary basement membrane as compared to the adult (Betz and Goldstein, 1979), the propensity of these vessels to rupture is high.

There is functioning sympathetic innervation in the cerebral circulation of the newborn piglet within two weeks of birth (Wagerle and Delivoria-Papdopoulos, 1987). In neonatal piglets and puppies however, most regional vascular beds have poorly

functional alpha adrenergic sympathetic supply at birth (Busija et al, 1985) and that the sensitivity of this system only increases to mature levels after several weeks.

Regional cerebral circulatory control by the sympathetic nervous system has been shown in the neonate by histochemical and pharmacologic studies (Busija et al, 1985; Edvinsson, 1975; Wagerle and Delivoria-Papadopoulos, 1987). Because of a differential concentration of alpha-adrenergic receptors in the carotid and basilar portions of the Circle of Willis (Edvinsson, 1975), there is the possibility of intracerebral shunting during periods of sympathetic stimulation.

Whether or not the cerebral sympathetic system is fully functional in a neonate less than 12 hrs of age is still a matter for speculation. Busija et al (1985) have shown pial artery constriction in response to sympathetic stimulation in 1-day-old piglets, and Wagerle and Delivoria-Papadopoulos (1987) have shown that postganglionic neuron and neuromuscular synaptic transmission mechanisms are functional at 2 days of life in piglets. In newborn piglets the superior sympathetic ganglion is functionally developed but sympathetic activation does not increase over the first weeks of life. Should the sympathetic system be functional, shunting of blood could lead to abnormal transmural stresses in certain vascular beds, and should the sympathetic system be immature, the lack of a moderating force would allow unchecked surges of pressure into the cerebral circulation.

It is unlikely that a MAP increase of 45% would alone account for the pathology seen in this study. Fluctuations in systolic pressure of the order of 100% (McDonald et al, 1984; Kontos et al, 1978) and fluctuations in cerebral blood flow velocity (Perlman and Volpe, 1983) have been shown to be associated with intraventricular haemorrhage. The act of laryngoscopy and intubation must therefore induce intracerebral changes of a much greater magnitude than would be expected on the basis of a pure MAP increase. As will be discussed later these changes may be linked to the release of vasoactive neurotransmitter substances which induce various degrees of vascular contraction and dilatation, and may also cause changes in vessel wall protein permeability. Increases in the permeability of cerebral blood vessels has been reported following the antidromic stimulation of branches of the trigeminal nerve (Moskowitz et al, 1988). Increased protein extravasation into the interstitial tissues following a hypertensive surge may lead to worsened cerebral oedema in an already distressed neonate, and contribute to the ultimate picture of cerebrovascular damage.

Cogniscence must be taken of the fact that the insertion of a foreign object into brain substance may have altered the brain blood flow dynamics. It is however unlikely that a localised peripheral event, with cortical penetration of only 2mm, could have been responsible for such extensive lesions, but this criticism of the methodology is valid, and should be taken into account when assessing the reported histology.

It is interesting to note that, following this experiment, all of the animals recovered from the anaesthetic, and that those animals who were intubated did not behave in any grossly different manner postoperatively to those anaesthetised and monitored. Yet, there was obvious histologic evidence of non-specific, non lethal bleeding in the basal area of the brain in 10 out of 11 intubated animals.

The results of the phase 1 experiment suggest that in hypercarbic neonatal piglets the stimulus of prolonged laryngoscopy and/or endotracheal intubation is sufficient to induce significant intracranial injury. This injury has been shown to include cerebral haemorrhage, intraparenchymal as well as intraventricular (specifically 4th ventricle and central canal), cerebral oedema and extravascular protein extravasation. Whether or not the same stimulus causes similar pathology in normoxic and normocarbic animals has not been addressed in this study. It may well be that the hypercarbia played a major role in the generation of these bleeds, and that the laryngoscopy/intubation was simply the precipitating event. Nevertheless the data have shown significantly less pathology in the Control group which was exposed to the same hypercarbic conditions as the Intubation group, and this evidence strongly supports the conclusion that the laryngoscopy/intubation was ultimately responsible for the observed findings. The fact that a single animal in the Control group bled is of concern, but because of the nature and distribution of this bleed, and the fact that the animal in

question was exposed to a significant hypertensive episode, the likely explanation of this single haemorrhage is on the basis of a non laryngotracheal sympathetic stimulus.

It is possible that in human neonates there are similar bleeds resultant from endotracheal intubation which are relatively "silent" at the neonatal period and may only present at a later stage with behavioral and minimal brain dysfunction syndromes.

Often in a resuscitation situation the neonate has a combination of hypercarbia and acidosis prior to intubation, and the noxious stimulus associated with laryngoscopy and intubation may contribute to the development of intracerebral pathology. The frequency with which neonatal endotracheal intubation is performed, and the potential damage with which it may be associated, as suggested by the findings of this preliminary study, were deemed sufficient to justify further more definitive investigation of the effects of laryngoscopy/intubation in the neonate.

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1 Sample groups - Phase 2

Although there were initially 20 animals entered into this phase of the study, 3 animals were excluded from each group. In all cases this was due to anaesthetic related complications.
The mean weight of the animals in the Saline group was 1.60 +/- 0.2 kg and in the Lignocaine group, 1.58 +/- 0.07 kg.

2 Monitored parameters - Phase 2

2.1 Blood pressure

2.1.1 Systolic

Although there were initially 10 animals in each group the statistics have been calculated from two groups of 7 animals. In 2 cases animals collapsed during the initial phase of anaesthesia and were resuscitated, thus excluding them from the study. In a further 2 cases technical difficulty with the monitoring equipment prevented the aquisition of usable data. Two animals were inadvertantly incinerated by a laboratory assistant before tissue specimens could be harvested for microsphere estimations.

The systolic blood pressure readings shown in Table P2-1 were read directly from the computer drawn graphs of the left ventricular pressure trends.

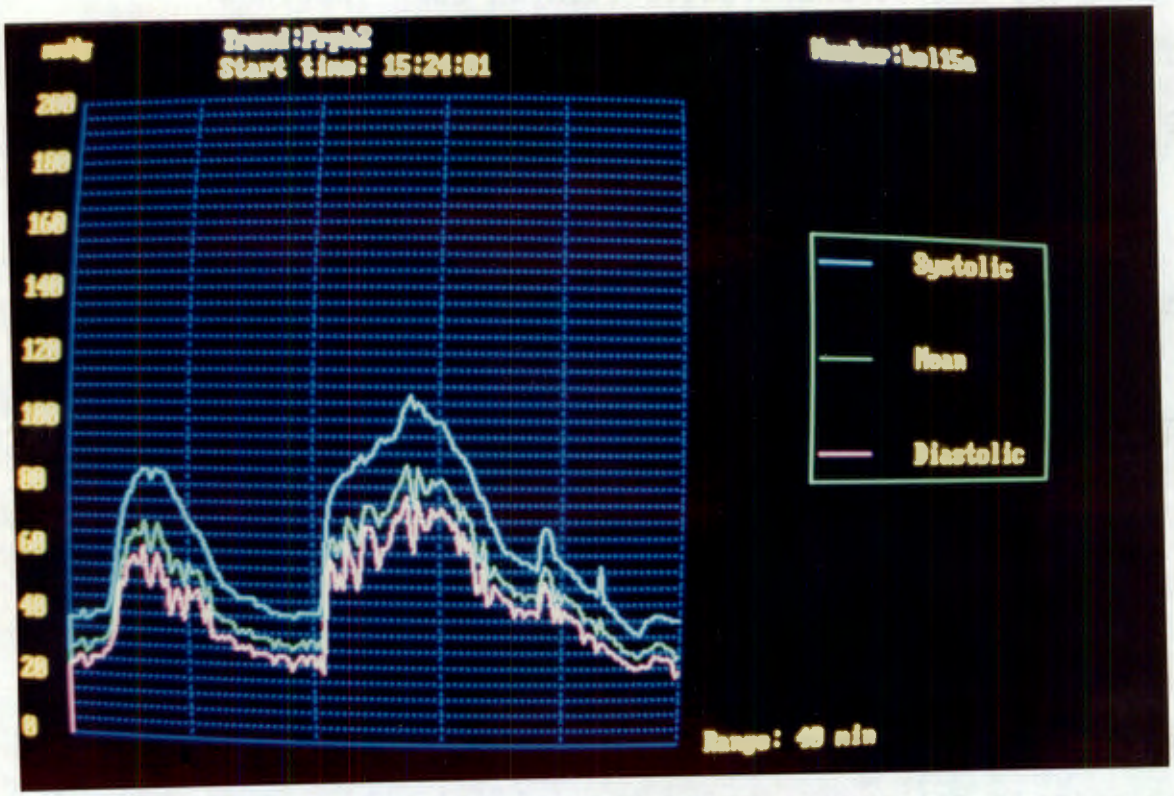


Figure P2-1: Screen display showing the response to laryngoscopy/ spray, and laryngoscopy/intubation in an animal sprayed with saline. Note the more pronounced and more prolonged pressure response following laryngoscopy/intubation.

TABLE P2-1: Systolic blood pressure changes following laryngoscopy/spray, and laryngoscopy/intubation.*

	SALINE GROUP (n = 7)	LIGNOCAINE GROUP (n = 7)	p
PRE-SPRAY	64 +/- 13 mmHg	66 +/- 14 mmHg	NS
PEAK-SPRAY	87 +/- 11 mmHg#	90 +/- 12 mmHg#	NS
PRE-INTUBATION	64 +/- 13 mmHg	66 +/- 11 mmHg	NS
PEAK-INTUBATION	95 +/- 11 mmHg+	82 +/- 13 mmHg$	<0.05

*Data presented as: mean +/- standard deviation

NS = No significant difference between the two groups (p = 0.05)

Significantly different from the Pre-spray level (p<0.01)

$ Significantly different from the Pre-intubation level (p<0.05)

+ Significantly different from the Pre-intubation level (p<0.01)

Least significant difference = 13.2 (p=0.05), 17.6 (p=0.01)

Figure P2-1 is an example of such a graph.

There were no significant differences in systolic pressure in either the saline or the lignocaine groups before, or within 5 minutes of laryngoscopy and spraying. In both groups the systolic pressure was equally and significantly ($p < 0.01$) raised during laryngoscopy and spraying. During laryngoscopy and intubation the systolic pressure was significantly raised in both groups
($p < 0.01$), however in the Lignocaine group this increase was less than in the Saline group ($p < 0.05$).

2.1.2 Mean

Although pressure traces of peripheral pressures were recorded, there were technical problems with the catheters. As a result, in very few animals was it possible to acquire continuous peripheral traces from which a mean peripheral blood pressure could be calculated. For this reason the left ventricular pressures were used in the analysis.

2.1.3 Pulse pressure

The pulse pressure analysis is presented in Table P2-2.

TABLE P2-2: Pulse pressures at baseline, during laryngoscopy/spray, and during laryngoscopy/intubation.

	SALINE GROUP (n=4)	LIGNOCAINE GROUP (n=5)	p
BASELINE (mmHg)	28 +/- 9	29 +/- 10	NS
INTRA-LARYNGOSCOPY/ SPRAY (mmHg)	40 +/- 5#	44 +/- 8#	NS
INTRA-LARYNGOSCOPY/ INTUBATION (mmHg)	41 +/- 4#	45 +/- 9#	NS

*Data presented as: Mean +/- standard deviation.

NS = No significant difference between the two groups (p=0.05)

Significantly different from baseline level (p<0.05)

Least significant difference = 10.8 (p = 0.05)

The pulse pressures are shown to widen during the time of peak stimulation. Because of small sample group size a meaningful analysis is not possible, but there is definitely a trend. There were no differences between the groups at these times. A more detailed analysis is presented in the phase 3 discussion.

2.1.4 Time-to-peak/Time-to-baseline

The time taken to reach the peak systolic pressure following laryngoscopy/spraying and laryngoscopy/intubation, and the time taken to return to the baseline value again are shown in Table P2-3.

TABLE P2-3: Time-to-peak (T-T-P) systolic pressure and time-to-baseline (T-T-B) for Saline and Lignocaine groups following laryngoscopy/spray (L/S) and laryngoscopy/intubation (L/I).*

	SALINE GROUP (n = 7)	LIGNOCAINE GROUP (n = 7)	p
T-T-P L/S (minutes)	4.2 +/- 4.6	6.1 +/- 3.4	NS
T-T-P L/I (minutes)	4.9 +/- 2.9	4.0 +/- 1.0	NS
T-T-B L/S (minutes)	10.9 +/- 9.5	9.4 +/- 6.0	NS
T-T-B L/I (minutes)	11.6 +/- 6.7	10.6 +/- 4.1	NS

* Data presented as: Mean +/- standard deviation

NS = No significant difference between the two groups (p=0.05)

Least significant difference for Time to peak = 3.5 (p=0.05)

Least significant difference for Time to baseline = 7.1 (p=0.05)

There were no statistically significant differences in the time-to-peak or time-to-baseline values after the laryngoscopy/ spray. Following laryngoscopy/intubation both the time-to-peak systolic pressure and time-to-baseline systolic pressure were less in the Lignocaine group, but these differences were also not significant.

These results indicate that the time taken to reach the peak blood pressure and the time taken to return to the baseline following laryngoscopy/spraying and laryngoscopy/intubation are similar in both groups, and that lignocaine spray does not obviously modify these time responses.

2.2 Cardiac Output

The cardiac output was calculated by standard methods using the microsphere technique (Heymann, 1977). The results are shown in Table P2-4.

TABLE P2-4: Cardiac output change during laryngoscopy/ intubation.*

	SALINE GROUP (n=7)	LIGNOCAINE GROUP (n=7)	p
PRE-LARYNGOSCOPY/ INTUBATION (ml/min/100g)	101 +/- 27	99 +/- 21	NS
INTRA LARYNGOSCOPY (ml/min/100g)	232 +/- 76#	249 +/- 93#	NS

*Data presented as: Mean +/- standard deviation.

NS = No significant difference between the two groups (p=0.05)

ml/min/100g = millilitres of blood per minute per 100 grams of tissue

#Significant difference from pre-laryngoscopy/intubation (p<0.01)

Least significant differences = 69.1 (p = 0.05)

= 93.5 (p = 0.01)

Laryngoscopy/intubation induced a statistically significant increase in cardiac output in both groups ($p < 0.01$). The difference between the 129% increase in the Saline group and the 152% increase in the Lignocaine group was not significant.

2.3 Heart rate

2.3.1 Tachycardia/bradycardia

Heart rate was calculated continuously by the computer from the arterial waveform. The results are presented in Table P2-4. Because of an error in the storage program only 6 cases were analysable, and this data is presented in full. Of note are the two cases that became bradycardic during intubation, one in either group. In neither case was there any evidence of hypoxia (PaO_2 > 300 mmHg in both cases). Severe bradycardia was not seen during laryngoscopy alone, and the precipitating event in both cases was the introduction of the endotracheal tube. Because the microsphere injection was completed more than 10 seconds before the occurance of the dysrhythmia in each case the blood flow rates derived from the microsphere injections have been included. Bradycardic response to endotracheal intubation will be discussed later.

TABLE P2-5: Heart rate response in 6 animals following laryngoscopy/spray and laryngoscopy/intubation.

	SALINE GROUP			LIGNOCAINE GROUP		
LARYNGOSCOPY/SPRAY						
ANIMAL NO.	1	2	3	4	5	6
BASELINE (bpm)	130	125	125	135	155	135
PEAK (bpm)	155	145	155	155	180	155
T-T-P (minutes)	1	2	4	2	2	1
T-T-B (minutes)	8	7	8	6	2	10

Figure P2-2: Heart rate response following laryngoscopy/ intubation. The arrow marks the beginning of the stimulus. The deep dips marked by symbols indicate microsphere injections.

Figure P2-3: Note the bradycardic response seen in this animal both during the laryngoscopy/spray, and following intubation.

LARYNGOSCOPY/INTUBATION

ANIMAL NO.	1	2	3	4	5	6
BASELINE (bpm)	120	125	135	135	155	135
PEAK (bpm)	220	75	155	160	182	155
T-T-P (minutes)	2	2	5	3	4	3
T-T-B (minutes	20	2	8	12	4	8

T-T-P = Time to peak heart rate

T-T-B = Time to baseline heart rate

Figure P2-2 shows a typical heart rate response as recorded by the computer trend plotter. Figure P2-3 shows an example of a bradycardia following intubation.

2.3.2 Dysrhythmias

Dysrhythmias of note encountered during the experiment, apart from those mentioned above, were ventricular extrasystoles, and on occasion, runs of ventricular tachycardia.

These perturbations in heart rate were usually seen during the induction phase of the anaesthetic. Care was taken to avoid injection of microspheres until a regular heart rate was re-established.

Laryngoscopy alone did not result in changes in heart rate other than tachycardia. The introduction of the endotracheal tube caused bradycardia, as mentioned above, in two cases. In none of the animals were there any ventricular extrasystoles or runs of ventricular tachycardia during laryngocopy or intubation.

2.4 Peripheral vascular resistance

Table P2-6 presents the data for the calculated peripheral vascular resistance.

TABLE P2-6: Peripheral vascular resistance at baseline, and during laryngoscopy/intubation.*

	CONTROL GROUP (n=4)	LIGNOCAINE GROUP (n=4)	p
+ BASELINE	50 +/- 6	40 +/- 4	NS
INTRA-LARYNGOSCOPY/ INTUBATION	33 +/- 11#	21 +/- 7#	<0.05

*Data presented as: Mean +/- standard deviation.

NS = No significant difference between the two groups (p=0.05)

+ units of measurement = mmHg.min.100 grams of tissue

Significantly different from baseline level

Least significant difference = 11.0 (p = 0.05)

This data has been derived from the cardiac outputs and mean arterial pressures that were available. The following formula was used:

$$\text{Peripheral vascular resistance} = \frac{\text{Mean arterial pressure}}{\text{Cardiac output}}$$

Because of the technical difficulty in obtaining and recording an accurate central venous pressure, this parameter was unfortunately not measured. The central venous pressure range in normal neonateal piglets is not well reported, but data derived from work in this laboratory shows baseline levels of between 0 and 4 mmHg. The contribution to the calculation is thus minimal, and unless there were very large fluctuations in central venous pressure (which was unlikely in the absence of coughing and straining), the derived vascular resistance values may still be regarded as indicative of the trend.

2.5 Blood gases

2.5.1 pH

Table P2-7 shows the blood gas status of the two groups of animals.

TABLE P2-7: Blood gas parameters following laryngoscopy/spray and laryngoscopy/intubation.*

	SALINE GROUP	LIGNOCAINE GROUP	p
BASELINE pH	7.22 +/- 0.07	7.30 +/- 0.07	NS
POST L/S pH	7.26 +/- 0.09	7.30 +/- 0.14	NS
POST L/I pH	7.29 +/- 0.07	7.29 +/- 0.13	NS
BASELINE PO2	281 +/- 90	251 +/- 57	NS
POST L/S PO2	247 +/- 114	246 +/- 104	NS
POST L/I PO2	224 +/- 133	242 +/- 103	NS

BASELINE PCO2	59 +/- 12	52 +/- 13	NS
POST L/S PCO2	54 +/- 14	51 +/- 27	NS
POST L/I PCO2	49 +/- 10	51 +/- 19	NS

---

*Data presented as: Mean +/- standard deviation

NS = Not significant at p = 0.05

L/S = Laryngoscopy/spray

L/I = Laryngoscopy/intubation

Least significant difference for pH = 0.11 (p = 0.05)

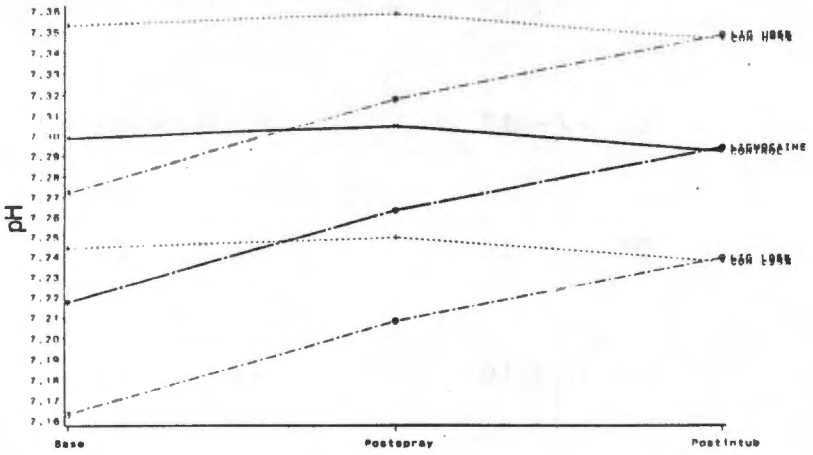
Least significant difference for O2 = 111 (p = 0.05)

Least significant difference for CO2 = 18 (p = 0.05)

The pH of both groups remained constant throughout the experiment and there were no differences that achieved statistical significance at a p=0.05 level. When the blood flow rates of the two groups were compared, the baseline and post spray blood flow rates in the Saline group appeared uniformly higher than in the Lignocaine group. There were however, no significant differences by analysis of variance, but the higher blood flow rates may be related to the lower pH and higher PaCo2 values.

pH

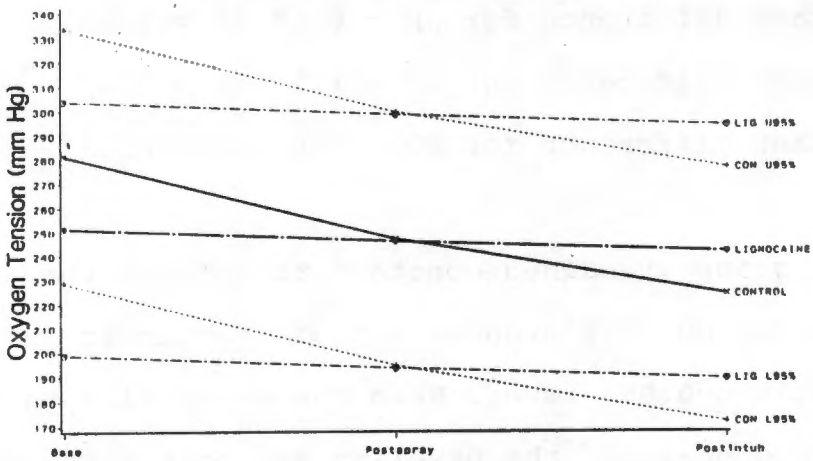
Means and their 95% confidence intervals.



Time points

Oxygen Tension

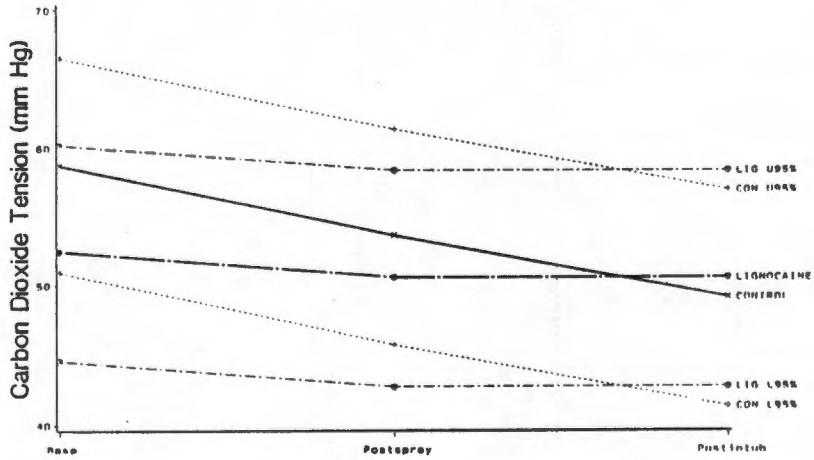
Means and their 95% confidence intervals.



Time points

Carbon Dioxide Tension

Means and their 95% confidence intervals.



Time points

2.5.2 PaCO2

The arterial PaCO2 levels did not vary significantly during the interventions, either between the groups or within the groups. The baseline PaCO2 in both groups was within the hypercarbic range and was most probably a result of rebreathing during the anaesthetic induction. It should be noted that the PaCO2 did not change significantly during the interventions, and that although baseline cerebral blood flow estimations in both groups of animals were undoubtedly higher than in normocarbic piglets, the rapid changes noted during this experiment are unlikely to be related to fluctuating arterial carbon dioxide levels.

2.5.3 PaO2

The animals were breathing a mixture of oxygen and Halothane and The stimuli did not cause hypoxia and there were no statistical differences between any of the estimations. All animals showed significant hyperoxia and this aspect of the experiment will be reviewed in the discussion.

3 Microsphere measurements

3.1 Number of microspheres in the tissues

As previously mentioned the number of microspheres in each tissue specimen determines the validity of any data derived therefrom. The critical number of 400 must be exceeded for the data to be meaningful (Heymann, 1976), and in this series of experiments the only tissue that did not contain more than 400 microspheres per estimation was the choroid plexus. In the upper cervical area the minimum number of microspheres per estimation was 750, while in all other regions the microsphere count was always in excess of 1000. In tissue specimens of larger mass such as the cerebral grey matter the number of microspheres counted was always in excess of 20 000.

3.2 Regional brain blood flow

3.2.1 Regional brain blood flow-% change

Table P2-8 details the %change in regional brain blood flow at 5 minutes after the laryngoscopy/spray, and again following the laryngoscopy/intubation, in the 11 areas of interest.

Because of sub-optimal numbers of microspheres in the tissues the choroid plexus has either been excluded from the following tables, or included only for completeness sake - the values are not to be regarded as reliable.

Of note is the fact that the baseline absolute blood flow rates correlated well with those reported by Hansen et al (1984) for neonatal piglets within this PaCO2 range.

TABLE P2-8: Cerebral blood flow analysis post laryngoscopy/spray and intra laryngoscopy/intubation. Data are presented as % change from baseline.*

		POST L/S (% CHANGE)	POST L/I (% CHANGE)
CEREBRAL GREY	- Saline	13 +/- 29	-31 +/- 17+
	- Lignocaine	-4 +/- 30	18 +/- 18#
CEREBRAL WHITE	- Saline	7 +/- 19	-33 +/- 16+
	- Lignocaine	-9 +/- 34	17 +/- 14#
UPPER CERVICAL	- Saline	-19 +/- 22	-50 +/- 12+
	- Lignocaine	1 +/- 50	3 +/- 29#

PONS	-	Saline	-11 +/- 24	-49 +/- 10+
	-	Lignocaine	-5 +/- 42	17 +/- 36#
MEDULLA	-	Saline	-19 +/- 21	-49 +/- 12+
	-	Lignocaine	-3 +/- 43	4 +/- 25#
MIDBRAIN	-	Saline	-12 +/- 18	-49 +/- 12+
	-	Lignocaine	2 +/- 51	3 +/- 32#
CEREBELLUM	-	Saline	-5 +/- 13	-37 +/- 25+
	-	Lignocaine	-13 +/- 40	8 +/- 25#
CAUDATE	-	Saline	-5 +/- 18	-26 +/- 21
	-	Lignocaine	-2 +/- 45	15 +/- 21#
THALAMUS	-	Saline	-5 +/- 20	-42 +/- 11+
	-	Lignocaine	-7 +/- 38	27 +/- 35+#
CHOROID	-	Saline	28 +/- 54	-27 +/- 37+
	-	Lignocaine	7 +/- 44	1 +/- 26#

HIPPOCAMPUS	-	Saline	-6 +/- 18	-37 +/- 18+
	-	Lignocaine	-4 +/- 36	13 +/- 13#

*Data presented as: Mean +/- standard deviation

+Significantly different from baseline value in same group (p=0.05)

#Significant difference between the Saline and Lignocaine groups (p=0.05)

Least significant difference = 31.5 (p = 0.05)

= 41.6 (p = 0.01)

Table P2-9 shows the %change in blood flow at the same time points but in this table the tissue specimens making up the 4 major divisions of the brain have been grouped together.

TABLE P2-9: Cerebral blood flow analysis post laryngoscopy/spray (L/S) and intra laryngoscopy/intubation (L/I).*

			5 MINUTE POST L/S (% CHANGE)	POST L/I (% CHANGE)
CEREBRUM	-	Saline	10 +/- 24	-32 +/- 16+
	-	Lignocaine	-6 +/- 32	18 +/- 16#
MIDBRAIN	-	Saline	-7 +/- 18	-38 +/- 15+
	-	Lignocaine	-3 +/- 42	14 +/- 25#

BRAIN STEM -	Saline	-16 +/- 23	-49 +/- 11+
-	Lignocaine	-2 +/- 45	7 +/- 33#
CEREBELLUM -	Saline	-5 +/- 13	-37 +/- 25+
-	Lignocaine	-13 +/- 40	8 +/- 25#

*Data presented as: Mean +/- standard deviation

+Significantly different from baseline value in same group (p=0.05)

#Significant difference between the Saline and Lignocaine groups (p=0.05)

Least significant difference = 31.5 (p = 0.05); 41.5 (p = 0.01)

There were no significant changes in blood flow between the two groups at the 5 minute post laryngoscopy/spray measurement, in any of the 11 brain regions (Table P2-8). These findings indicate that:

1) the post laryngoscopy/spray blood flow rates were not significantly different from the baseline levels in either group at 5 minutes after the stimulus,

2) there was no significant difference in the % change in the blood flow rates between the two groups at the post laryngoscopy/spray measurement,

3) there was no significant inter regional shunting in either the Saline or Lignocaine groups resultant from the laryngoscopy/spray, and

4) there was no significant shunting effect at the 5 minute post laryngoscopy/spray estimation in any of the brain regions resultant from a lignocaine effect.

3.3 Total brain blood flow

Table P2-10 shows the %change in total brain blood flow 5 minutes after laryngoscopy/spray, and during laryngoscopy/intubation.

TABLE P2-10: Total brain blood flow analysis 5 minutes post laryngoscopy/spray and during laryngoscopy/intubation.

	POST LARYNGOSCOPY/ SPRAY (% CHANGE)	POST LARYNGOSCOPY/ INTUBATION (% CHANGE)
SALINE	-6 +/- 20	-43 +/- 20

LIGNOCAINE	-4 +/- 41	13 +/- 25

---

*Data presented as Mean +/- standard deviation

Least significant difference = 31.5 (p = 0.05)

All statistically significant results are based on a p value of 0.05.

There were no significant changes from the baseline blood flow at 5 minutes post laryngoscopy/spray in either group, and nor was there any difference in the direction or magnitude of the change between the 2 groups.

Following laryngoscopy/intubation there was a significant decrease in total brain blood flow in the Saline group, while the Lignocaine group showed an increase which was not statistically significant. In addition there was a significant difference in %change in total brain blood flow between the two groups.

3.4 Organ blood flow

Table P2-11 shows the % change in organ blood flow from the baseline level in each group, following laryngoscopy/intubation.

TABLE P2-11: Table showing the % change in organ blood flow from baseline to post laryngoscopy/intubation in the Saline and Lignocaine groups.*

ORGAN	SALINE GROUP % CHANGE	LIGNOCAINE GROUP % CHANGE	p
ADRENAL	-14 +/- 23	23 +/- 21	< 0.05
KIDNEY	-5 +/- 41	25 +/- 20	NS
MASSETER	30 +/- 42	13 +/- 41	NS
LIVER	-17 +/- 48	12 +/- 53	NS
SPLEEN	5 +/- 80	-5 +/- 40	NS
LEFT VENTRICLE	-31 +/- 28	38 +/- 97	NS
LUNG	-13 +/- 20	16 +/- 44	NS

* Data presented as: Mean +/- standard deviation

NS = Non significant by analysis of variance

Although in most cases there appeared to be a trend to decreased organ blood flow in the Saline group, and increased organ blood flow in the Lignocaine group following the stimulus, only the adrenal gland showed a statistically significant difference.

4 Summary of cardiovascular and cerebrovascular results

The results presented from this phase of the study indicated the following points:

1. Irritation of the larynx, pharynx and trachea is a potent stimulus and caused significant increases in blood pressure, both during laryngoscopy/spray and laryngoscopy/intubation.

2. Pulse pressure and cardiac output were also increased during the peak stimulus period.

3. There were no significant differences in the time intervals, either in the time taken to reach the peak pressure, or the time taken to return to the baseline pressure.

4. Heart rate also increased, but because of small sample size a detailed analysis was imposible. It is important to note that the oft reported bradycardia during intubation did not occur in all cases, and only one animal showed a heart rate of less than 100 beats per minute in response to intubation.

5. Laryngoscopy/intubation was shown to reduce peripheral vascular resistance during the peak response period, with the lignocaine treated animals appearing to exhibit lower peripheral vascular resistances.

6. Lignocaine spray was shown to significantly reduce the peak systolic pressure response to laryngoscopy/intubation.

7. There were no significant differences in the blood gases either within or between the two groups during the experiment, indicating that there was minimal effect on the blood flow rates that could be attributed to blood gas changes.

8. In the control animals laryngoscopy/intubation was shown to significantly reduce the regional brain blood flow rates in all brain regions during the stimulus, while in the lignocaine treated animals there was an insignificant change in brain blood flow. Because only a single blood flow rate estimation was made in this series of experiments it was not possible to acquire a full picture of the dynamic changes following the stimulus, and whether this apparent difference represented a delayed response, or a different response, was not discernable from this data. For this reason the phase 3 study was designed.

5 Discussion Phase 2

The data presented represent the first prospective controlled blinded study of the cardiovascular and cerebrovascular changes following laryngoscopy and endotracheal intubation in term neonates. In addition, this is the first prospective blinded study of the use of local anaesthetic spray in the reduction of the cardiovascular and cerebrovascular perturbations resulting from such intervention in term newborns.

Stoelting (1977) found that in adults the majority of the blood pressure response to laryngoscopy and intubation was caused by the laryngoscopy, with a small additional increase in pressure being noted following passage of the endotracheal tube. From the present study these pressure changes are shown to occur in newborns (piglets) as well. The reduced blood pressure response to laryngoscopy/intubation in the Lignocaine group demonstrates a potential use for the drug in situations where acute elevation of systolic blood pressure would be disadvantageous.

Following the laryngoscopy/spray there was a blood pressure response that was equal in both groups. The time intervals were also very similar. After the laryngoscopy/intubation there were significant changes in systolic arterial blood pressure in both groups. The Saline group showed an increase greater than that seen following laryngoscopy/spray, and in the Lignocaine group the increase was less than that seen after laryngoscopy/spray. Had the groups been larger these intra-group differences may have

achieved statistical significance. The peak systolic pressure seen in the Lignocaine group was however significantly less than that in the Saline group.

This data is interesting on two counts. Firstly, the difference in the increase in blood pressure seen after the laryngoscopy/ intubation in the Saline group seems to reflect the greater degree of stimulation applied to the larynx and pharynx. Whether this is the result of the added irritation of the endotracheal tube, or the result of the increased duration of the laryngoscopy is not clear. The significantly smaller blood pressure increase seen in the Lignocaine group following laryngoscopy/intubation must be explained on the basis of reduced peripheral vasoconstriction since there was an equal increase in cardiac output in both groups. This may have been a result of a vasodilator effect of lignocaine, (Goodman and Gilman, 1975). Denlinger et al (1974) suggested that tracheal intubation is accompanied by sympathoadrenal stimulation additional to that of laryngoscopy alone, and that this additional stimulus could be blocked by the intra-tracheal spray of lignocaine 120mg/70kg, allowing 5 minutes for surface analgesia to take effect. The present study may be interpreted as confirming these findings exactly in newborn piglets. Whether the effect of the lignocaine is mediated by surface analgesia or systemic action, is less easy to confirm. Data in this study indicate that if the lignocaine does have a systemic action, this is most likely to be one of peripheral vasodilatation, since the cardiac output change was unaffected by the drug during laryngoscopy/intubation. Without

adequate data it is difficult to assess the contribution of the heart rate changes to the cardiovascular response, but the relationship is certainly complex. This aspect will be covered later.

The pulse pressure increased during laryngoscopy/intubation (see figure P2-1), but there were no significant differences between the two experimental groups (Table P2-2). This increase in pulse pressure may be explained on the basis of the decreased peripheral vascular resistance associated with the laryngoscopy/intubation (see Table P2-6). This probably represents a vasomotor centre mediated attempt to alleviate the sudden increase in blood pressure resulting from the sympathetic neural stimulation. In this situation the sympathetic vasodilator fibres to the peripheral muscles are activated by the anterior hypothalamus, via the mesencephalon and sympathetic preganglionic neurons in the lateral horns of the cord (Guyton, 1980). Increased cervical sympathetic activity was noted during irritation of the respiratory tree by Tomori and Widdicombe (1969), supporting the sympathetic vasodilator hypothesis. Sympathetic stimulation has also been shown to increase the cardiac output and heart rate, and strength of the contraction of the heart (Guyton, 1980). All of these changes would contribute to an increase in stroke volume and an increase in pulse pressure. In addition there may be a contribution from the increased heart rate during peak stimulation. Although this is one of the less important determinants of pulse pressure, Guyton

(1980) states that rapid onset of heart contraction sometimes occurs when a heart is beating vigourously, and this causes large increases in the pressure within the aorta before blood can run off into the peripheral circulation. Therefore sudden ejection causes a greater pulse pressure than does more prolonged ejection.

Both groups showed significant increases in cardiac output following laryngoscopy/intubation. There was no difference in the degree of the increase between the Saline and the Lignocaine groups, implying that the lignocaine did not cause myocardial depression during the procedure ($p<0.01$).

Since the cardiac output was measured at the peak of the stimulation, the vascular resistance at this time is unlikely to show the effects of sympathoadrenal ouflow which would tend to cause vasoconstriction - adrenal medulla activity would be represented by a delayed response with vasoconstriction and elevated blood pressure. The decrease in peripheral vascular resistance preceded the development of the maximum blood pressure response (peak blood pressure at 4.2 minutes), suggesting that the vasodilatation was mediated by a different system, most probably neural. As is shown later in the phase 3 results, the vasodilatation is followed by an increase in the peripheral vascular resistance which is concurrent with the development of the maximal blood pressure and may represent catecholamine induced vasoconstriction.

The possibility of re-opening of the ductus arteriosus during these periods of stress should not be forgotten in a newborn animal model. Although unlikely this may lead to reduced vascular resistance. One of the reasons for maintaining oxygen tension at a high level in this study was to prevent functional opening of the ductus arteriosus. Moss et al (1963) showed that in newborns 100% oxygen consistently closed the ductus arteriosus within the first 12 hours of life, and that withdrawal of the 100% oxygen led to a reopening of the ductus. During this study the animals were kept on 100% oxygen throughout ensuring functional ductal closure, and it is unlikely that the pulse pressure increase can be explained on this basis. Work by Laptook et al (1982) has shown that there is no shunting from right to left across the ductus arteriosus in newborn piglets.

The time taken to attain the peak blood pressure levels was similar (in both groups) after the laryngoscopy/intubation, to that seen following laryngoscopy/spray. The difference in the time taken for the blood pressure to return to baseline levels was not significant between the two groups, and was similar to that seen after the laryngoscopy/spray.

The delayed nature of the pressure increase (the peak blood pressure was attained well after the stimulation was ended), the duration of the response, and the time taken for the blood

pressure to return to within 5 mmHg of the baseline level are all suggestive of a humoral response. Stimulation of the respiratory tree causes the release of catecholamines (Derbyshire et al, 1983) and this may explain the humoral effects seen here.

The data from this study suggest that the predominant neural effect of laryngoscopy and endotracheal intubation is in fact sympathetic, and not parasympathetic. The cardiac output and heart rate increase (shown in phase 3) associated with a decrease in peripheral vascular resistance and a widened pulse pressure, are all indicative of increased sympathetic nervous activity. These findings are significant since they may indicate a dual control of the response to laryngoscopy/intubation - an initial neural response, not shown to be affected by the lignocaine, and as demonstrated later, a secondary humoral response probably altered by the lignocaine.

The heart rate increased in all of the six animals for whom data is available during the laryngoscopy/spray, and in 5 of the 6 animals during laryngoscopy/intubation. In the animal that had a decrease this was transient and occurred during the intubation (Figure P2-3). This animal was one of the Saline group and there was no evidence of hypoxia during this decrease in heart rate. The usual explanation for decreases in heart rate during laryngoscopy, laryngeal spraying and endotracheal intubation has been on the basis of vagal stimulation (Mirakhur, 1982). Kelly and Finer (1984) suggest that in neonates the cardiovascular

response to intubation more closely parallels the response in the newborn lamb with laryngeal chemoreceptor stimulation elicited by the injection of water into the larynx. They also compare the response to the trigeminal diving reflex in lambs. There are however some important differences shown in this study that make this laryngeal chemoreceptor hypothesis unlikely. The newborn lamb, in response to laryngeal chemoreceptor stimulation, develops apnoea, hypertension, bradycardia and a decrease in cardiac output proportional to the reduction in heart rate, and an increase in systemic vascular resistance (Grogaard and Sundel, 1983). The data in the present study show an increase in heart rate and cardiac output, and a decrease in systemic vascular resistance during laryngoscopy/intubation; a totally different picture to that suggested by Kelly and Finer (1984). These authors quoted Friedman (1972) in the explanation of the findings of their study in which decreases in heart rate associated with laryngoscopy and intubation were accompanied by hypertension. Friedman (1973) reported that the fetus had a severely restricted capability for making compensatory changes in stroke volume due to immaturity of the myocardium and a lack of sympathetic innervation to the ventricles. Data from the present study show this not to be the case in newborn piglets in whom heart rate and cardiac output increases are very much in evidence. Pressor and depressor loci have been found within the hypothalamus, pons, and medulla of newborn piglets (Gootman et al, 1972). The distribution of these sites and the responses associated with them, which are not obviously different from the adult, show that

both generalised and regionally restricted changes in sympathetic outflow can be evoked. As discussed earlier, the majority of studies reporting decreases in heart rate with intubation have not excluded hypoxia as a contributory influence. This aspect must always be considered.

Transient bradycardia in the face of normal oxygen tension may be explained on the basis of a baroreceptor response. Baroreceptor sensitivity increases after birth and it has been well demonstrated that newborn lamb heart rate slows when arterial pressure is increased (Shinbourne et al, 1972; Dawes et al, 1980). Consistent changes in heart rate are only obtained with relatively large (10-15 mmHg) increases in arterial pressure (Dawes et al, 1980). The decrease in the heart rate in one of the 6 animals reported on in this phase of the study may be related to a baroreflex.

As has been discussed in the introduction, there is little good data to indicate that lignocaine spray is of use in attenuating the cardiovascular response to laryngoscopy/intubation in adults. The reason for its effectiveness in this study may be explained to a large extent by the fact that the spray was performed at least 10 minutes before the stimulation began. This allowed adequate time for the drug effect to develop, either by analgesia of the mucosa, or by systemic absorption.

Since the microsphere injection was started after initiation of the laryngoscopy, the blood flow rate changes seen at the laryngoscopy/intubation estimation may have been representative of an already established autoregulatory response. It was possible that an increase in blood flow during the initial stages of the stimulus was missed by measuring the flow rate at a time when there may have already been a degree of cerebral vasoconstriction in the Control group. This indicated the need for a longitudenal study of brain blood flow changes that incorporated earlier measurement of the brain blood flow rates, as well as a longer follow-up of the ensuing changes after the stimulus.

Because of large inter animal differences and small sample group size it is difficult to analyse absolute tissue flows without a high probability of type 2 statistical error, and for this reason the %change in the regional brain blood flow rates has been used in the analysis (Table P2-8). There were no significant differences in the baseline absolute blood flow rates between the two groups.

At the 10 minute post laryngoscopy/spray brain blood flow measurement there were no statistically significant %changes from the baseline values in either of the two groups even though the lignocaine would have been absorbed and circulating at this time (Bromage and Robson, 1961). This implies that at a time when there is minimal sympathetic stimulation lignocaine has little effect on the control of cerebral blood flow. Because the 10

minute post laryngoscopy/spray level should be regarded as the new baseline, the analysis of the blood flow changes has been made using this reset level.

When the baseline and 10 minute post laryngoscopy/spray absolute tissue flows were compared, there was a constant trend for the Saline group to have insignificantly higher regional brain blood flow rates. This may be explained by the slightly higher PaCO2 values and lower pH levels seen in the Saline group. These blood gas differences were more pronounced during the baseline estimations than at any of the other measurements, and by the time the post laryngoscopy/intubation estimations were made the values were almost identical. If the values are corrected (2.4% increase in cerebral blood flow for each 1mmHg increase in PaCO2 (Reivich, 1964)) the flow rates are very similar at both baseline and 10 minutes post laryngoscopy/intubation.

In the Saline group the laryngoscopy/intubation was followed by an increase in blood pressure and cardiac output and a decrease in cerebral blood flow, both total and regional, without any evidence of selective regional shunting. In the Lignocaine group, there were no significant changes in the regional brain blood flow during the laryngoscopy/intubation, indicating a probable drug effect. These regional changes were mirrored in the total brain blood flow rates, with similar insignificant %changes at the 10 minute post laryngoscopy/spray estimation, and a significantly decreased %change in the Saline group as opposed to the Lignocaine group, during the laryngoscopy/intubation. The

intense stimulus of the laryngoscopy/intubation was associated with an equal increase in the cardiac output in the two groups, but a lower peak blood pressure in the Lignocaine group. The calculated peripheral vasodilatation was also lower in the Lignocaine group. Relaxation of the vascular muscle in the peripheral muscles by the lignocaine, combined with sympathetic vasodilatation, may have accounted for the lower peripheral resistance in this group when compared to the Saline group. Numbers are small and differences do not achieve statistical significance, but the trend does appear to support this hypothesis.

The explanation of why the regional brain blood flow decreased in the Saline group and not in the Lignocaine group might lie in the different blood pressure responses experienced by the two groups. The autoregulatory function of the cerebral vasculature is well described (Heistad and Kontos, 1984) and within the physiological range of pressure and cardiac output, cerebral blood flow is known to be strictly maintained. Regional brain blood flow in the Saline group may have exceeded the physiological limits and stimulated cerebral vascular reflexes, which then caused protective constriction of the cerebral vessels. In the Lignocaine group, a number of possibilities exist. Because of the lower blood pressure at the time of the post laryngoscopy/intubation brain blood flow estimation, the blood flow may have still been within the physiological limits and had not yet stimulated autoregulatory cerebral vasoconstriction.

This lower blood pressure may have resulted from reduced sympathetic stimulation at a local level, or from a systemic vasodilatory effect of the lignocaine.

The effect of the lignocaine on the cerebral blood vessels themselves must also be considered. Data is however contradictory. Lignocaine has been shown to significantly increase brain blood flow (57%) during seizures in animals (Sakabe et al, 1974; Plum et al, 1968), and this increase is not entirely related to passive increases due to raised mean arterial pressure. Lescanic et al (1981) have shown that lignocaine in lower doses than those used by Sakabe et al (1974) actually causes cerebral vessels to vasoconstrict, and this finding has been demonstrated in other vascular tissue by various workers (Cibils, 1976; Klein et al, 1968; Altura, 1967). Kurth et al (1988) have shown that seizures have a potent sympathetic stimulatory effect, and it may be that the increased brain blood flow shown by Sakabe et al (1974) was the result of the sympathetic vasodilatation caused by seizures induced by toxic doses of lignocaine, rather than from any intrinsic effect of the drug itself. At a metabolic level Sakabe et al (1974) also showed that seizures in lignocaine treated animals increased the cerebral metabolic rate for oxygen by 12%. This increased metabolic rate for oxygen may have caused a change in the local cellular millieu leading to a decrease in cerebrospinal-fluid oxygen, and consequent cerebral arteriolar vasodilation by a

local metabolic mechanism. There does not appear to be any significant blood brain barrier for lignocaine (Sakabe et al, 1974) and the drug easily passes into the brain.

Available evidence suggests that lignocaine in non-toxic doses is a cerebral vasoconstrictor, and that in this phase 2 experiment, by reducing the surge of blood flow during the stimulation, the lignocaine prevented the blood flow rate from surpassing the threshold level for "switching on" the cerebral autoregulation. Since the blood flow rate was still within the autoregulation limits it was possible that the flow rate may have increased insignificantly above the baseline level. It is highly unlikely that the lignocaine totally prevented the cerebral blood flow surge, but by delaying the surge slightly (by a direct cerebral vasoconstrictor effect), and by lowering the cerebral perfusion pressure (reducing the mean arterial pressure by a peripheral vasodilator effect), it is likely that the post laryngoscopy/ intubation measurement in phase 2 was too early to document the autoregulatory vasoconstriction in the Lignocaine group. In the Saline group however, normally reactive cerebral vessels and a higher mean arterial pressure at the time of measurement ensured that autoregulation was present, as evidenced by the significantly reduced cerebral flow rate.

The physiological role of sympathetic nerves arising from the superior cervical ganglion have recently been shown to play an important role in the regulation of cerebral blood flow under

certain circumstances such as acute hypertension or during hypercapnia (Heistad and Kontos, 1984). They may serve to regulate intravascular pressure in the cerebral microvasculature, and protect against sudden increases in blood pressure. It is posible that the lignocaine in some way potentiated a vasoconstrictive response, or reduced a possible vasodilatatory response, of the cerebral vessels to the sympathetic stimulation induced by laryngoscopy.

A further intriguing possibility is based on the differential distribution and rate of development of sympathetic receptors in the neonatal period. This model would explain the seemingly incongruous responses of the systemic and cerebral circulations to the laryngoscopy/intubation, viz systemic vasodilatation associated with cerebral vasoconstriction in the Saline group, and more pronouced systemic vasodilatation, and possibly increased cerebral vasoconstriction, in the Lignocaine group. Alpha-adrenergic cerebral vasoconstrictor mechanisms are present and functionally developed in newborn piglets (Busija et al, 1985; Wagerle and Delivoria-Papadopoulos, 1987). In addition greater concentrations of alpha-adrenergic nerve terminals are present on cerebral arteries derived from the carotid portion of the Circle of Willis than on the basilar portion (Edvinsson, 1975). Therefore in neonates blood flow to the cerebral grey tissue, the caudate nucleus and the thalamus, supplied by the

carotid circulation, would be more affected by sympathetic vasoconstriction than blood flow to the brain-stem and cerebellum which are supplied by the basilar circulation.

In contrast to the cerebral circulation, most other regional vascular beds have immature alpha-adrenergic function at birth (Buckley, 1986; Buckley et al, 1986). Thus it is possible that in times of sympathetic stimulation the cerebral circulation would be relatively more sensitive to alpha-adrenergic stimulation than the peripheral circulation. This would allow relative overactivity of the beta-adrenergic receptors in the peripheral muscles with vasodilatation and decreased peripheral vascular resistance. The addition of lignocaine could then be seen to potentiate peripheral vasodilatation, and potentiate cerebral vasoconstriction, as occurred in this experiment.

The changes in brain blood flow were not synchronous with the changes in blood pressure and this suggests that the reflex cerebral vessel changes were possibly initiated by a separate mechanism to the blood pressure changes. This reinforces the hypothesis that there are immediate effects of laryngoscopy/intubation (increased heart rate and cardiac output, associated with systemic sympathetic vasodilatation, and cerebral sympathetic vasoconstrition) which are most likely to be neurally mediated, and delayed effects (peripheral vasoconstriction and increasing blood pressure) that are humorally regulated.

The Lignocaine group showed a lower peripheral resistance at the 10 minute post laryngoscopy/spray estimation suggesting a drug effect. In addition there was a significantly lower peripheral resistance during laryngoscopy/intubation in the Lignocaine group than in the Saline group, which may explain the difference in blood pressure between the two groups at this time. The lower peripheral vascular resistance in the Lignocaine group during laryngoscopy/intubation was almost certainly the result of a systemic vasodilator lignocaine effect combined with the sympathetic vasodilator effect.

Tissue blood flow was also analysed with %change values. There were only two tissues in which significantly different %change values were obtained viz., adrenal and masseter. In both of these tissues blood flow following laryngoscopy/intubation in the Saline group was shown to be significantly less than in the Lignocaine group. This aspect of the study was not pursued.

The findings in this phase of the study were confusing in that they showed a definite insult effect, and a definite modification of this effect by the treatment. The restricted number of observations did not permit any real analysis of the detailed cerebral blood flow changes during and following the laryngoscopy and endotracheal intubation, and for this reason phase 3 was planned.

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1 Phase 3 - Reiteration of the aims

The phase 3 experiment was designed to add data to that gained from the phase 2 study. Phase 3 investigated the same cardiovascular and cerebrovascular parameters, but with some important timepoint differences. In phase 3 the cardiovascular effects were documented over a longer time period, allowing a more detailed picture of the return to baseline intervals. The major differences between phase 2 and phase 3 in terms of the cerebral blood flow measurements were threefold:

Firstly, the "laryngoscopy/intubation" shoot was carried out at an earlier stage in the laryngoscopy/intubation stimulus than in phase 2, and provided data on blood flow rates at a slightly earlier timepoint after initiation of the stimulus than in phase 2.

Secondly, the greater number of timepoints in phase 3 allowed more detailed study of the brain blood flow patterns.

Finally, the fact that there had been minimal preparatory stimulation of the larynx in the phase 3 animals (as opposed to laryngoscopy/spray in the phase 2 study) tended to reduce the confounding variable of previous sympathetic stimulation which may have already sensitised the cerebral vasculature, and changed the response pattern.

From the phase 2 findings it appeared that blood flow rates in all brain regions in the Control group were significantly reduced below baseline levels following laryngoscopy/intubation, and that this change was not evident in the Lignocaine group. It became obvious that some form of cerebral autoregulation was responsible for this vasoconstriction, and it seemed likely that an earlier blood flow estimation might demonstrate the surge of blood flow causing the compensation. The finding of a functioning cerebral autoregulatory system was significant, since it has been suggested that hypercarbia desensitises cerebral autoregulation (Lou et al, 1979). The effect of the prior laryngoscopy on the cerebral vasculature in phase 2 could not be excluded, and the need for blood flow estimations bracketing the stimulus period was apparent. Because of the constraints of the measurement technique it was not possible to perform repeated blood flow estimations in the critical immediate post stimulus time period, and the design of the phase 3 study was a compromise based on these limitations.

Since differences in experimental design did not allow extrapolation of the phase 2 post laryngoscopy/intubation results into the phase 3 post laryngoscopy/intubation analysis, the phase 3 study design was such that only data from the baseline readings in phase 2 was used. Justification of the experimental design has been previously addressed in this thesis.

2 Sample groups - Phase 3

Although 18 animals were initially entered into the study there were a number of technical problems which excluded 6 piglets from the microsphere experiment - 2 cases of presumed malignant hyperthermia, 1 case of sudden cardiac arrest following microsphere injection, 1 case in which there was excessive bleeding during the insertion of the axillary artery catheter, and 2 cases in which reference blood samples were unsatisfactory. In these latter 2 cases, all other measured parameters have been included in the analysis.

The mean weight of the animals in the Control group was 1.76 +/- 0.1 kg and in the Lignocaine group 1.82 +/-0.11 kg.

3 Monitored Parameters - Phase 3

3.1 Blood Pressure

3.1.1 Systolic

The peak systolic pressure changes induced by the various stimuli in phase 3 are shown in Table P3-1.

TABLE P3-1: Systolic blood pressure changes following laryngoscopy/intubation (L/I) in the Control group, and spray and laryngoscopy/intubation (L/I) in the Lignocaine group.*

	BASELINE (mmHg)	PEAK (mmHg)	p
CONTROL GROUP (n = 7)	64 +/- 15	96 +/- 16	< 0.01
LIGNOCAINE GROUP (n = 7)			
SPRAY	60 +/- 11	74 +/- 12	< 0.05
L/I	60 +/- 8	74 +/- 12	< 0.05

*Data presented as: Mean +/- standard deviation

The peak systolic pressure increased significantly following laryngoscopy/intubation (L/I) in both groups. In the Control group there was a 52 +/- 23% increase ($p<0.01$) and this was a significantly greater increase than that seen in the Lignocaine group (24 +/- 14%). The peak systolic pressure increase seen during laryngoscopy/intubation (L/I) in the Lignocaine group was similar to that noted during the lignocaine spray, and although this was a significant change from the baseline ($p<0.01$) the increase was markedly less ($p<0.01$) than that shown following laryngoscopy/intubation (L/I) in the Control group.

3.1.2 Mean

Table P3-2 details the peak mean arterial pressure response in the two groups. There was no significant difference in the baseline values and the changes mirror those seen for the systolic pressure. The peak mean arterial pressure change following laryngeal spray in the Lignocaine group was less significant ($p<0.05$) than that seen after laryngoscopy/intubation (L/I) in the Control group ($p<0.01$), while the peak mean arterial pressure increase following laryngoscopy/intubation (L/I) in the Lignocaine group was not significant at the $p<0.05$ level.

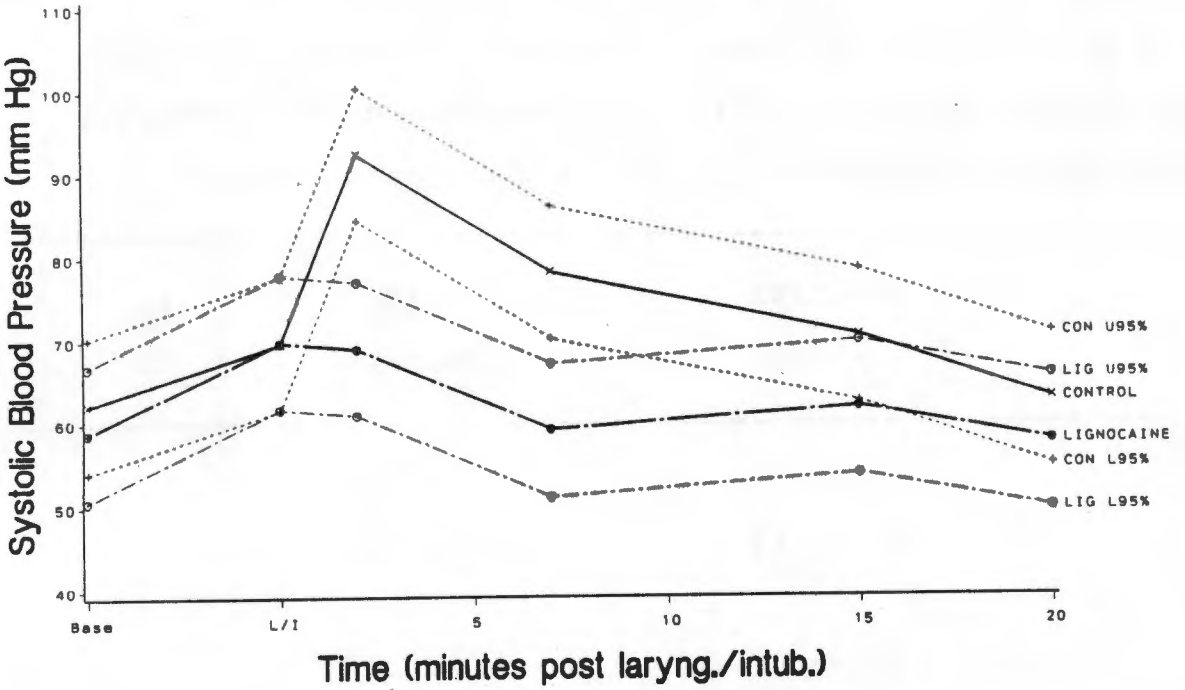
TABLE P3-2: Mean arterial pressure changes following laryngoscopy/intubation (L/I) in the Control group and spray and laryngoscopy/intubation (L/I) in the Lignocaine group.*

	BASELINE (mmHg)	PEAK (mmHg)	p
CONTROL GROUP (n = 7)	45 +/- 14	67 +/- 12	< 0.01
LIGNOCAINE GROUP (n = 7)			
SPRAY	42 +/- 6	54 +/- 10	< 0.05
L/I	45 +/- 9	54 +/- 8	NS

*Data presented as: Mean +/- standard deviation

Systolic Blood Pressure

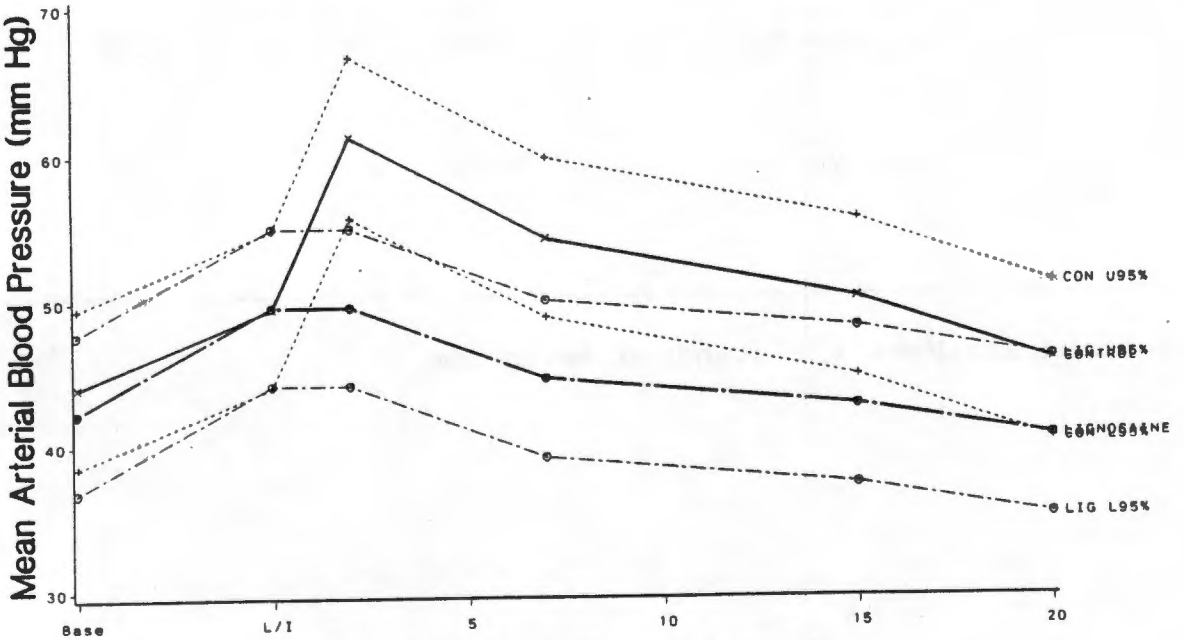
Means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Mean Arterial Blood Pressure

Means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

3.1.3 Blood Pressure at fixed timepoints

3.1.3.1 Systolic

Systolic pressure and mean arterial pressure levels measured at the same timepoints as the brain blood flow estimations are shown opposite. Analysis of the systolic pressure over the whole experimental period showed a highly significant difference between the two groups, with the Lignocaine group demonstrating a significantly lower systolic pressure ($p = 0.0001$). Pairwise analysis showed a significant increase in systolic pressure during intubation ($p = 0.0168$), and a 2 minute systolic pressure that was significantly higher than all other timepoints ($p < 0.004$). Between the two groups, however, there were significant differences at the 2 minute ($p = 0.0001$) and 7 minute timepoints ($p = 0.0006$) with the Lignocaine group showing a significantly lower systolic pressure.

Within the two groups multivariate analysis, using $p < 0.01$ as the level of significance, showed significantly elevated systolic blood pressure in the Control group at 2 minutes ($p = 0.0001$) and at 7 minutes (0.0036), whilst in the Lignocaine group the systolic pressure increases at the same timepoints were insignificant.

Note that in these graphs, the peak systolic and peak mean arterial pressure points are not displayed, falsely indicating that the 2 minute time point was the peak pressure point. The peak blood pressure points are tabulated on the previous pages in Table P3-1 and Table P3-2.

3.1.3.2 Mean

Analysis of the mean arterial pressure values at the fixed timepoints revealed very similar findings to those above. The mean arterial pressure over the whole time period was significantly less in the Lignocaine group ($p = 0.0002$). There were significantly lower values in the Lignocaine group at 2 minutes ($p = 0.002$) and 7 minutes ($p = 0.0089$), and even at 15 minutes there was still a marginal difference between the two groups ($p = 0.04$).

Within the Control group mean arterial pressure was significantly raised above the baseline value at 2 minutes ($p = 0.0001$), and 7 minutes ($p = 0.0055$). In the Lignocaine group mean arterial blood pressure was not significantly elevated at any of the measured timepoints, including the peak mean arterial pressure value.

3.1.4 Pulse Pressure

TABLE P3-3: Pulse pressures at baseline, during, and after the Laryngoscopy/intubation (L/I).

(mmHg)	SALINE GROUP (n=7)	LIGNOCAINE GROUP (n=7)	p
BASELINE	29 +/- 7	26 +/- 4	NS
INTRA-L/I	35 +/- 10	34 +/- 6	NS
2 MINUTES	41 +/- 8	32 +/- 4	NS
7 MINUTES	33 +-/ 5	28 +/- 7	NS
15 MINUTES	31 +/- 7	28 +/- 7	NS
20 MINUTES	31 +/- 7	26 +/- 5	NS

*Data presented as: Mean +/- standard deviation.

NS = Not significant at p = 0.05

Table P3-3 details the pulse pressure changes during the monitoring period.

3.1.5 Time-to-peak/Time-to-baseline

TABLE P3-4: Time-to-peak and time-to-baseline systolic pressure following laryngoscopy/intubation (L/I) and spray.*

	TIME-TO-PEAK (minutes)	TIME-TO-BASELINE (minutes)
CONTROL GROUP (n = 7)	3.8 +/- 1.5	12.5 +/- 3.8
LIGNOCAINE GROUP (n = 7)		
SPRAY	3.6 +/- 0.7	8.1 +/- 3.6 #
L/I	4.2 +/- 2.5	8.0 +/- 3.9 #

* Data presented as: Mean +/- standard deviation

Means significantly different from Control Group ($p < 0.001$).

Table P3-4 shows the time intervals for the systolic pressure changes following laryngoscopy/intubation in the Control group and spray and laryngoscopy/intubation in the Lignocaine group.

The time-to-peak systolic pressure was similar in all 3 situations with no differences of note. The group that had lignocaine took significantly less time to return to the baseline systolic pressure, regardless of the stimulus. These data strengthen the case for the trend noted in phase 2 in which there was a shorter time-to-baseline after laryngoscopy/intubation in the Lignocaine group.

3.2 Cardiac output

3.2.1 Flow rates

The changes in cardiac output following laryngoscopy/intubation in the two groups are shown in Table P3-5.

TABLE P3-5: Phase 3 cardiac output changes following laryngoscopy/intubation (L/I), with 2, 7, 15, and 20 minute values.*

	CONTROL GROUP (ml/min/100g) (n=6)	LIGNOCAINE GROUP (ml/min/100g) (n=6)	p
BASELINE	92 +/- 13	119 +/- 22	NS
INTRA-L/I	112 +/- 22	176 +/- 47#	NS
2 MINUTES POST L/I	220 +/- 64#	188 +/- 58#	<0.05
7 MINUTES POST L/I	119 +/- 31	156 +/- 54	NS
15 MINUTES POST L/I	125 +/- 31	155 +/- 40	NS
20 MINUTES POST L/I	174 +/- 31#	250 +/- 62#	<0.05

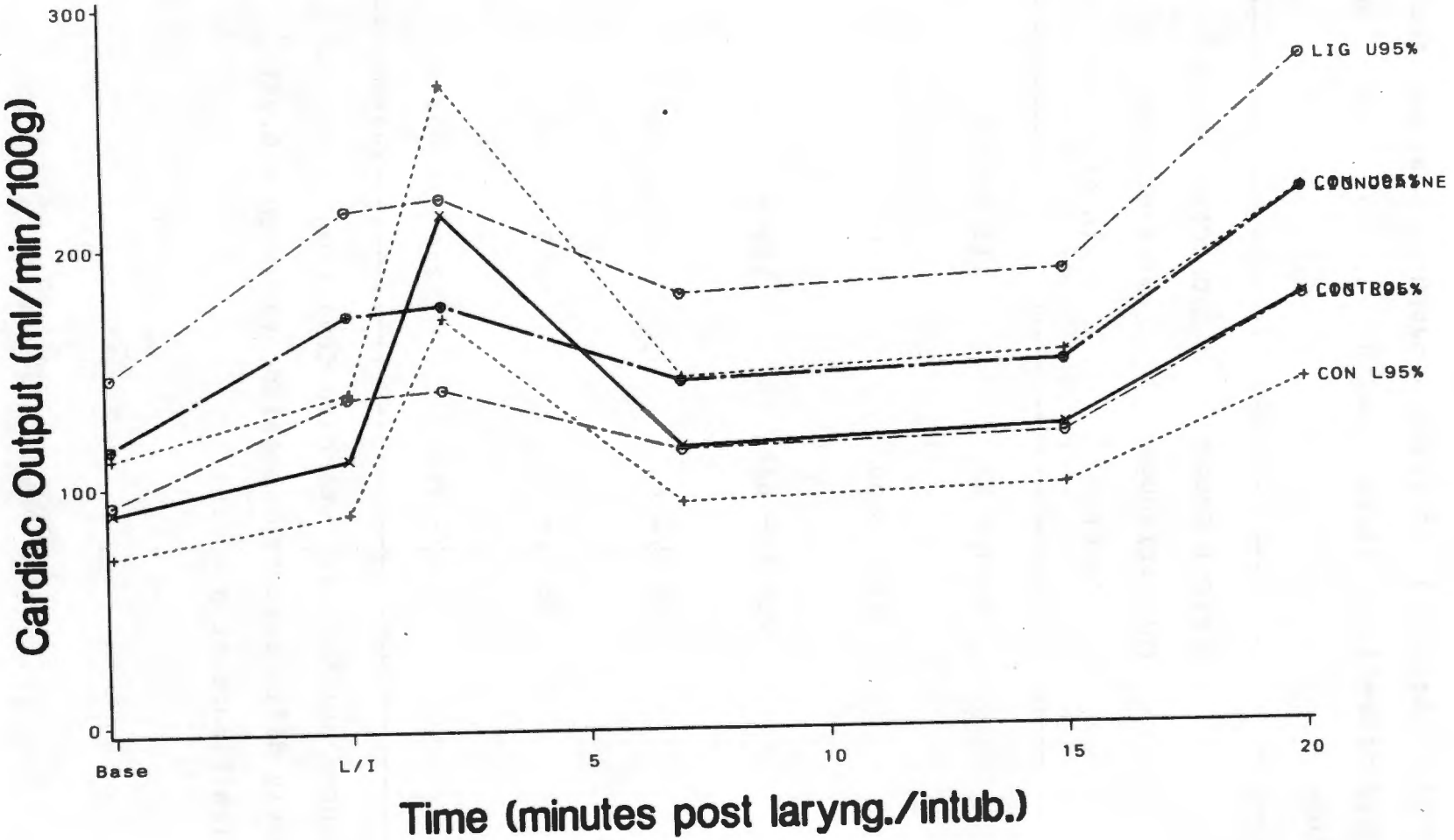
*Data presented as: Mean +/- standard deviation.

#Significantly differenet from baseline value (p = 0.05)

NS = Not significant at p = 0.05

Cardiac Output

Geometric means and their 95% confidence intervals.



Analysis of the cardiac output changes over the whole experimental period indicated that there was a significantly higher cardiac output in the Lignocaine group ($p = 0.0027$). This is an interesting finding since over the same time period the blood pressure in the Lignocaine group was significantly lower than that seen in the Control group. The explanation of this may be on the basis of a lower peripheral vascular resistance in the Lignocaine group, possibly a result of a peripheral vasodilatory drug effect. The cardiac output changes are shown graphically on the opposite page.

Pairwise comparison showed that the cardiac output was significantly elevated above baseline levels at all other times in the study ($p < 0.03$). Only in the Lignocaine group did the cardiac output increase significantly during laryngoscopy/ intubation (possibly indicative of a greater degree of peripheral vasodilatation in this group), but thereafter both groups showed significantly increased cardiac output at the 2 minute timepoint, and at the 20 minute timepoint (probably related to the hypercarbia).

The cardiac output did not at any time appear to have been depressed by the lignocaine.

3.3 Heart rate

3.3.1 Tachycardia/bradycardia

Table P3-7 shows heart rate responses to laryngoscopy/intubation in the Control and Lignocaine groups. The baseline values prior to laryngoscopy/intubation were similar in the two groups ($p = 0.38$). There was a significant increase in heart rate in both groups ($p<0.05$), but there was no significant difference in the peak heart rates between the two groups ($p = 0.2$).

Of the 14 animals studied for heart rate changes in phase 3 there were 2 that showed a decrease in heart rate following laryngoscopy. Only one of these animals developed a bradycardia (< 120 beats per minute) and in both cases the animals were from the Control group. The slowed heart rate was very short lived and was followed by an increase above the original baseline level as soon as the intubation began.

TABLE P3-7: Heart rate changes following laryngoscopy/intubation in the Control and Lignocaine groups.*

	BASELINE (beats per minute)	PEAK (beats per minute)	p
CONTROL GROUP (n = 7)	131 +/- 14	167 +/- 32	0.0007
LIGNOCAINE GROUP (n = 7)	138 +/- 13	157 +/-19	0.0156

*Data presented as: Mean +/- standard deviation

LI = Laryngoscopy/intubation

3.3.2 Time-to-peak/time-to-baseline

Table P3-8 tabulates the time intervals for the heart rate changes in phase 3.

TABLE P3-8: Time-to-peak heart rate and time-to-baseline heart rate following laryngoscopy/intubation in the two groups.

	TIME-TO-PEAK (minutes)	TIME-TO-BASELINE (minutes)	p
CONTROL GROUP (n = 7)	1.8 +/- 0.7	12.0 +/- 4.9	< 0.01
LIGNOCAINE GROUP (n = 7)	2.1 +/- 0.9	5.0 +/- 2.9#	< 0.05

*Data presented as: Mean +/- standard deviation

#Significantly different from Control Group (p = 0.05)

As was noted in the pressure response curves there was little difference between the two groups in terms of the time taken to reach the peak heart rate. There was however a significantly

shorter time taken to return to the baseline heart rate ($p < 0.05$) in the Lignocaine group.

3.3.3 Dysrhythmias

Dysrhythmias encountered during phase 3 were as seen in phase 2, i.e. ventricular extrasystoles, and on occasion, runs of ventricular tachycardia. These perturbations in heart rate were usually seen during the induction phase of the anaesthetic. Care was taken to avoid injection of microspheres until a regular heart rate was re-established.

Laryngoscopy alone did not result in changes in heart rate other than tachycardia, and as already mentioned, significant bradycardia in one case. The introduction of the endotracheal tube was associated with tachycardia in all cases. In none of the animals were there any ventricular extrasystoles or runs of ventricular tachycardia during laryngocopy or intubation.

3.4 Peripheral vascular resistance

The peripheral vascular resistance results before, during and after the stimulus are presented in Table P3-9. The results are shown graphically on the following page.

TABLE P3-9: Peripheral vascular resistance (mmHg/min/ml/100g of tissue) before, during, and after laryngoscopy/intubation.*

	CONTROL GROUP (n=6)	LIGNOCAINE GROUP (n=6)	p
+			
BASELINE	42 +/- 20	33 +/- 17	NS
INTRA-LARYNGOSCOPY/ INTUBATION	42 +/- 23	24 +/- 13	NS
2 MINUTES POST INTUBATION	28 +/- 15	26 +/- 15	NS
7 MINUTES POST INTUBATION	41 +/- 28	29 +/- 18	NS
15 MINUTES POST INTUBATION	38 +/- 24	28 +/- 19	NS
20 MINUTES POST INTUBATION	22 +/- 14	14 +/- 8	NS

*Data presented as: Mean +/- standard deviation.

NS = Not significant at p = 0.05

+ = mmHg/min/ml/100g of tissue

3.5 Blood gases

3.5.1 PaO2

All animals remained hyperoxic throughout the experimental period and there were no significant differences within or between the two groups when analysis of variance for repeated measurements was performed. The PaO2 and PaCO2 values are graphed on the opposite page.

3.5.2 PaCO2

There were no differences between the groups at the baseline or post laryngoscopy/intubation measuremnts and the PaCO2 did not vary significantly between these two times. The pre-sacrifice estimations were similar in both groups and were significantly higher than the baseline and post laryngoscopy/intubation levels.

TABLE P3-10: Phase 3 blood gas parameters at baseline, following laryngoscopy/intubation, and pre sacrifice (PRE-SAC), for the Control and Lignocaine groups of animals (n = 6).*

	CONTROL GROUP (n = 6)	LIGNOCAINE GROUP (n = 6)	p
BASELINE pH	7.22 +/- 0.03	7.26 +/- 0.08	NS
POST L/I pH	7.26 +/- 0.05	7.31 +/- 0.04	NS
PRE-SAC pH	7.18 +/- 0.09	7.24 +/- 0.05	NS
BASELINE PaO2	170 +/- 59	260 +/- 128	NS
POST L/I PaO2	145 +/- 107	243 +/- 93	NS
PRE-SAC PaO2	250 +/- 64	219 +/- 121	NS
BASELINE PaCO2	55 +/- 11	61 +/- 16	NS
POST L/I PaCO2	57 +/- 6	56 +/- 7	NS
PRE-SAC PaCO2	75 +/- 15 #	74 +/- 9 #	NS
BASELINE BE	-7 +/- 2	-4 +/- 2	NS
POST L/I BE	-7 +/- 8	-1 +-/ 1	<0.05
PRE-SAC BE	-7 +/- 4	-3 +/- 2	NS

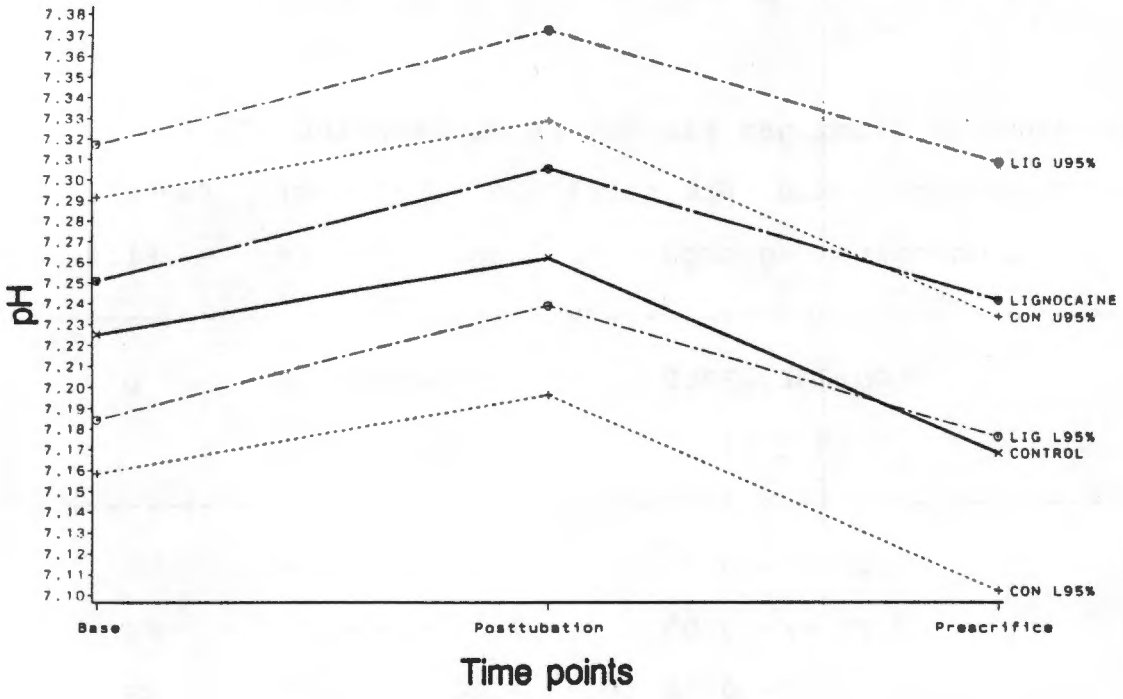
*Data presented as: Mean +/- standard deviation

#Value significantly different from baseline

NS = Not significant at p = 0.05

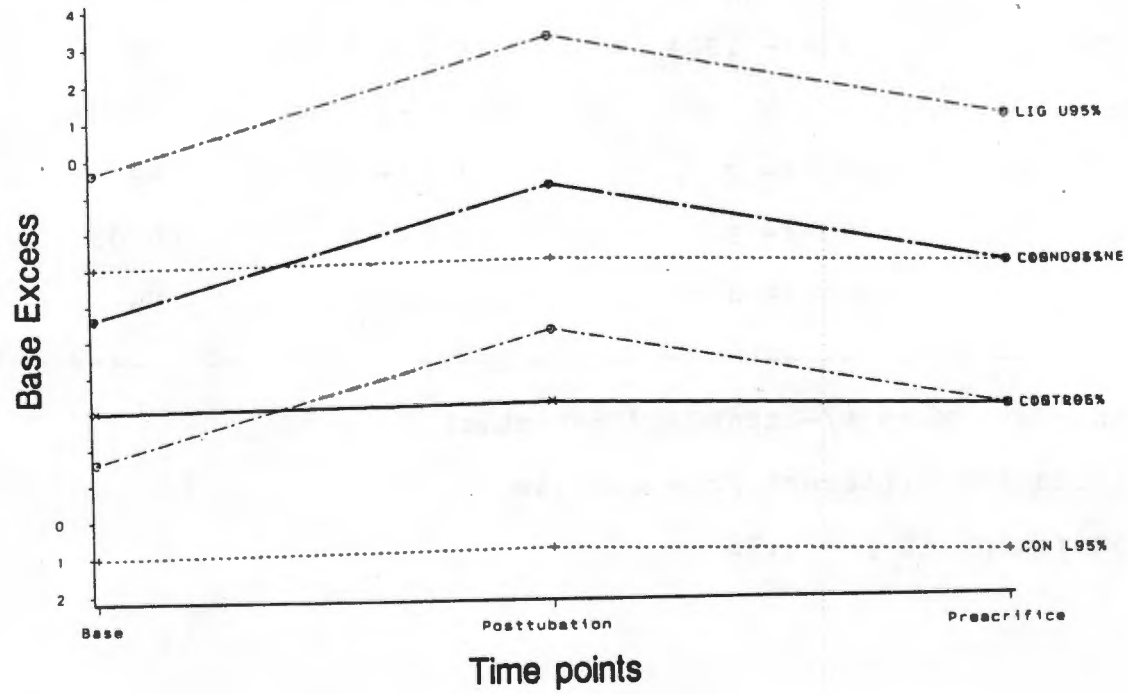
pH

Means and their 95% confidence intervals.



Base Excess

Means and their 95% confidence intervals.



L/I = Laryngoscopy/intubation, BE = Base Excess

3.5.3 pH

Table P3-10 shows the blood gas results for Phase 3.

Although all of the pH values in the Control group were lower than in the Lignocaine group these differences did not achieve significance. There were minimal alterations in both groups over the laryngoscopy/intubation period. In both the Control and the Lignocaine groups there were significant decreases in pH at the presacrifice estimation, and this correlates with the PaCO2 changes (below) and with other changes usually associated with acidaemia (increased cardiac output and increased regional cerebral blood flow). These results are similar to those seen in phases 1 and 2.
The results are displayed graphically on the opposite page.

3.5.4 Base Excess

The base excess values for the animals indicate that the Control group were significantly more acidaemic following the laryngoscopy/intubation than the Lignocaine group. There is a

trend for the lignocaine treated animals to have a smaller base deficit, and this may be related to the drug. There were no statistically significant changes within either group over the experimental period.

3.5.5 Analysis of co-variance

An analysis of co-variance was performed to demonstrate the effect, if any, of the blood gas changes, on the change in blood flow rates after laryngoscopy/intubation in the two groups. The effects of pH, PaCO2, PaO2 and Base Excess were assessed, and in no case were any significant effects noted. This finding is important since it demonstrates that the differences in the blood gas values between the two groups did not contribute significantly to the inter-group blood flow rate differences noted following the stimulus. Thus it may be assumed that the blood flow rate differences observed between the Control and Lignocaine groups following laryngoscopy/intubation, are resultant upon the drug effect rather than disparities in blood gas levels.

3.6 Oxygen Saturation

Figure P3-1: Peripheral oxygen saturation response to laryngoscopy/ intubation. The arrow marks the beginning of the laryngoscopy, and the diamond marks the intubation timepoint.

Table P3-11 details the changes in peripheral oxygen %saturation following the laryngoscopy/intubation. Baseline %saturation was comparable in both groups and the nadir values were at not less than 50%. The Control group had an insignificantly lower nadir value than the Lignocaine group. It may be that the lignocaine affected the cords in such a way as to reduce the amount of laryngospasm during laryngoscopy and in so doing reduced the decrease in the %saturation, but this is not strongly supported by the data. There was no difference between the groups in the time taken to return to the baseline value.

There was no change in peripheral oxygen saturation during the spraying of the larynx. This was the result of a gentle approach and the use of a narrow bore tube to deliver the aerosol spray.

Figure P3-1 shows an example of a typical peripheral oxygen saturation response to laryngoscopy/intubation.

TABLE P3-11: Phase 3 peripheral oxygen %saturation changes following laryngoscopy/intubation.*

	CONTROL GROUP (n = 7)	LIGNOCAINE GROUP (n = 7)
BASELINE (% saturation)	95 +/- 3	95 +/- 2
LOWEST LEVEL ATTAINED (% saturation)	74 +/- 6 +	79 +/- 10 +
TIME TO BASELINE (minutes)	2.3 +/- 1.1	2.4 +/- 0.8 #

*Data presented as Mean +/- standard deviation

+Significantly different from the baseline ($p < 0.01$)

#No statistical difference

4 Microsphere measurements

4.1 Number of microspheres in the tissues

As with the previous microsphere experiment the data for the choroid plexus measurements has been included for completeness sake, but because of the small quantities of tissue there were specimens that contained less than the requisite 400 microspheres. In all other cases there were more than 700 microspheres in each tissue specimen.

4.2 Regional brain flows and resistances

4.2.1 Baseline values

Table P3-12 shows the blood flow rates in ml/min/100grams tissue. The baseline blood flows were similar in both groups with no significant differences noted. The variances were large and the sample size small, again emphasising the possibility of a type 2 statistical error. There were no obvious differences between the two groups that reflected the blood gas trend in which the Control group appeared to have a more acidaemic baseline level. Blood flow to the basal regions of the brain (upper cervical, medulla, midbrain, pons) as well as to the thalamus, was higher than to the cerebrum (white and grey matter) at the baseline determination.

TABLE P3-12: Cerebral blood flow in ml/min/100g at the baseline (B/L) and post laryngoscopy/intubation (L/I).*

	B/L	L/I	2	7	15	20
		(minutes post laryngoscopy/intubation)				
CEREBRAL GREY						
Control#	74(29)	119(43)	94(24)	93(22)	109(27)	113(40)
Lignocaine#	80(25)	138(62)	74(20)	86(27)	105(33)	115(33)
CEREBRAL WHITE						
Control	108(44)	150(71)	137(77)	127(78)	110(22)	111(26)
Lignocaine	93(27)	125(55)	79(25)	95(35)	110(30)	121(31)
UPPER CERVICAL						
Control	344(251)	316(166)	254(123)	270(163)	264(99)	259(102)
Lignocaine	221(110)	163(55)	108(71)	146(92)	187(96)	207(120)

PONS						
Control	215(154)	262(166)	149(97)	195(121)	208(134)	204(99)
Lignocaine	175(94)	123(69)	97(53)	132(77)	178(93)	215(135)
MEDULLA						
Control	291(211)	289(112)	186(84)	252(86)	305(156)	287(130)
Lignocaine	277(106)	186(69)	127(55)	198(92)	245(73)	307(105)
MIDBRAIN						
Control	257(145)	297(166)	203(126)	243(130)	239(69)	228(71)
Lignocaine	223(89)	170(66)	127(53)	165(75)	227(93)	284(117)
CEREBELLUM						
Control	137(51)	179(66)	140(48)	152(42)	162(54)	167(48)
Lignocaine	124(36)	143(51)	103(34)	116(37)	143(36)	161(50)
CAUDATE						
Control	187(106)	223(105)	222(101)	205(123)	247(135)	237(112)
Lignocaine	241(104)	241(102)	170(77)	203(83)	273(155)	317(171)
THALAMUS						
Control	189(108)	221(95)	156(49)	173(33)	225(75)	227(88)
Lignocaine	189(71)	166(71)	112(40)	147(54)	198(71)	230(78)
CHOROID						
Control	-10 (36)	18 (41)	25 (71)	72(104)	-17 (23)	
Lignocaine	-12 (29)	-9 (36)	9 (52)	46 (95)	13 (21)	

HIPPOCAMPUS						
Control	151(122)	179(192)	134(130)	166(181)	115(51)	114(39)
Lignocaine	112(43)	104(32)	77(20)	95(27)	118(38)	135(43)

*Data presented as: Mean (standard deviation)

#n = 6

The subsequent changes in regional brain blood flow have been plotted and analysed both as absolute tissue flow rates, and as %change (Table P3-13).

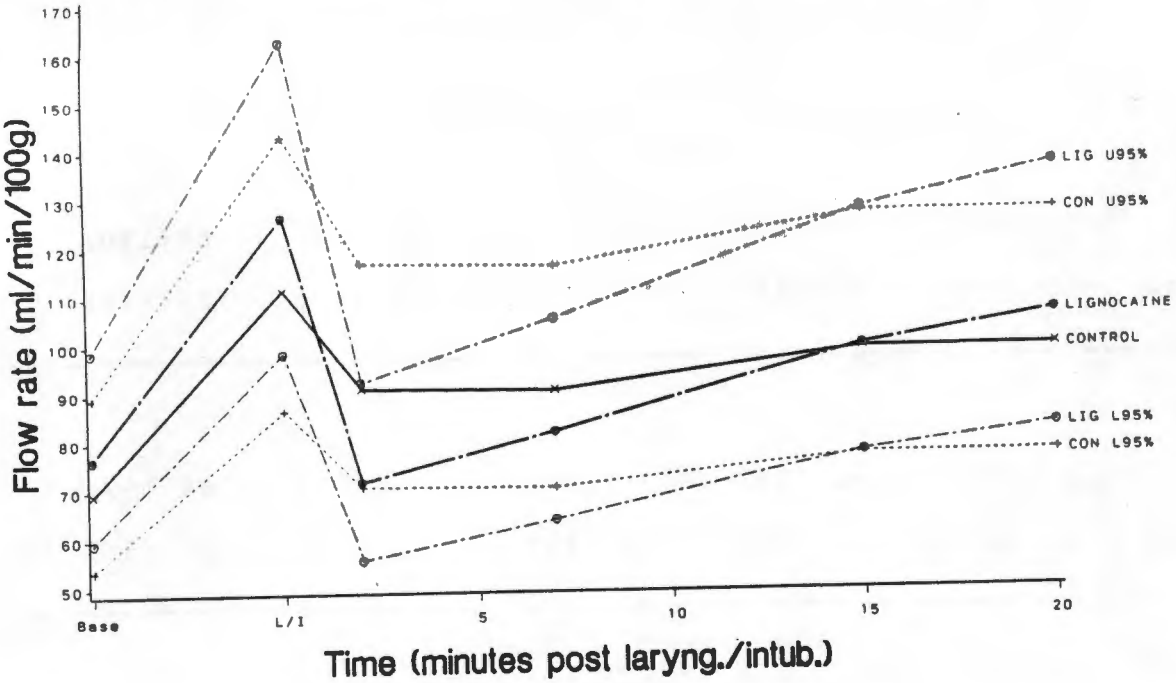
TABLE P3-13: Cerebral blood flow analysis post laryngoscopy/ intubation. Data presented as % change from baseline.*

	L/I	2	7	15	20
		(minutes post laryngoscopy/intubation)			
CEREBRAL GREY					
Control#	62 (15)	43 (35)	35 (37)	48 (48)	55 (73)
Lignocaine#	75 (67)	0 (41)	12 (32)	41 (57)	61 (77)
CEREBRAL WHITE					
Control	35 (13)	24 (31)	19 (38)	31 (46)	37 (66)
Lignocaine	31 (39)	-7 (37)	7 (36)	30 (55)	45 (64)
UPPER CERVICAL					
Control	14 (38)	-14 (48)	21 (64)	42 (90)	50(104)
Lignocaine	-41 (21)	-49 (19)	-29 (30)	-3 (42)	13 (47)
PONS					
Control	30 (28)	-14 (41)	20 (57)	41 (79)	50 (97)
Lignocaine	-16 (20)	-39 (29)	-20 (33)	11 (47)	40 (71)

MEDULLA					
Control	23 (41)	-7 (59)	33 (71)	51 (95)	57(110)
Lignocaine	-35 (24)	-49 (21)	-23 (37)	-1 (39)	22 (46)
MIDBRAIN					
Control	22 (22)	-5 (39)	21 (56)	39 (72)	48 (99)
Lignocaine	-15 (18)	-31 (30)	-15 (31)	12 (42)	37 (65)
CEREBELLUM					
Control	18 (34)	7 (29)	23 (43)	45 (73)	64 (83)
Lignocaine	26 (36)	-8 (46)	9 (42)	31 (76)	65(106)
CAUDATE					
Control	24 (25)	33 (39)	13 (43)	32 (41)	34 (55)
Lignocaine	-27 (27)	-19 (33)	-11 (26)	18 (50)	35 (48)
THALAMUS					
Control	26 (20)	0 (40)	22 (55)	40 (70)	49 (92)
Lignocaine	-11 (20)	-32 (30)	-15 (33)	14 (44)	38 (60)
CHOROID					
Control	-10 (36)	18 (41)	25 (71)	72(104)	-17 (23)
Lignocaine	-12 (29)	-9 (36)	9 (52)	46 (95)	13 (21)

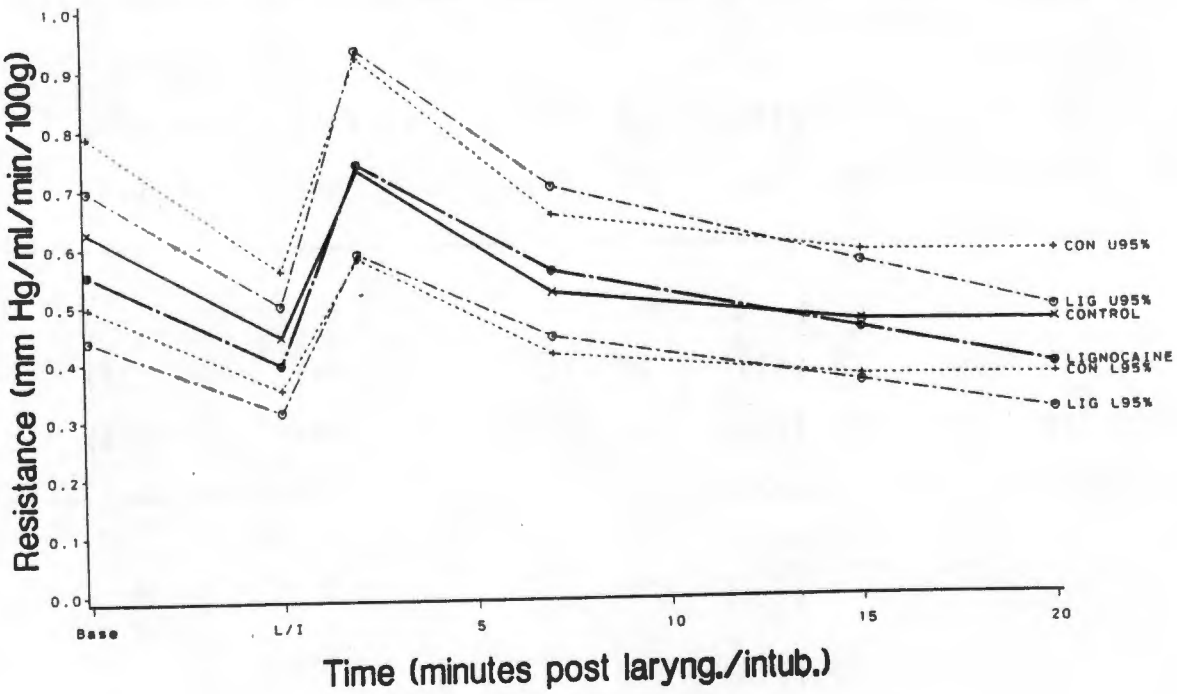
Cerebral Grey Matter - regional blood flow rate

Geometric means and their 95% confidence intervals.



Cerebral Grey Resistance.

Geometric means and their 95% confidence intervals.



HIPPOCAMPUS					
Control	7 (23)	-9 (34)	15 (50)	46 (83)	56(100)
Lignocaine	-11 (30)	-21 (35)	-7 (31)	14 (38)	34 (60)

*Data presented as: Mean (standard deviation)

L/I = Laryngoscopy/intubation

#n = 6

4.2.2 Flow rate and resistance

4.2.2.1 Cerebral grey

The blood flow rates and tissue resistances of the two groups were very similar. When the curves (opposite page)were analysed for differences over the entire study period, there was no significant treatment (lignocaine) effect on either blood flow or tissue resistance.

The baseline blood flow rate was similar to that noted in the cerebral grey matter of hyperoxic/normocapnic neonatal piglets (Wagerle and Delivoria-Papadopoulos, 1987).

Pairwise analysis of the blood flow at the different timepoints revealed that during laryngoscopy/intubation the blood flow was significantly higher than at the baseline ($p = 0.0001$), 2 minute ($p = 0.0015$), and 7 minute ($p = 0.0070$) timepoints regardless of treatment group. At no individual timepoints were there any significant differences in blood flow rate between the two groups.

There were statistically significant increases in blood flow rate seen within both groups during laryngoscopy/intubation ($p < 0.0054$).

As previously mentioned the cerebral grey tissue resistance changes did not show any significant drug effect when analysed over the whole study period ($p = 0.3378$). The two curves were essentially similar in shape.

In a pairwise comparison of tissue resistance at the various timepoints, regardless of treatment group, the 2 minute post stimulus tissue resistance was shown to be significantly higher than at all other timepoints ($p < 0.04$). In addition the tissue resistance at laryngoscopy/intubation was significantly lower than at the baseline estimation ($p = 0.0022$).

When the tissue resistances were examined for differences that could be ascribed to lignocaine effect, there were no significant differences at any of the timepoints. In addition, within each group there were no significant changes from the baseline at a $p = 0.01$ level.

The region of particular interest in this study has been selected as that time period during which the two groups were demonstrated to behave differently viz., the first 15 minutes, and in particular the time around the laryngoscopy/intubation.

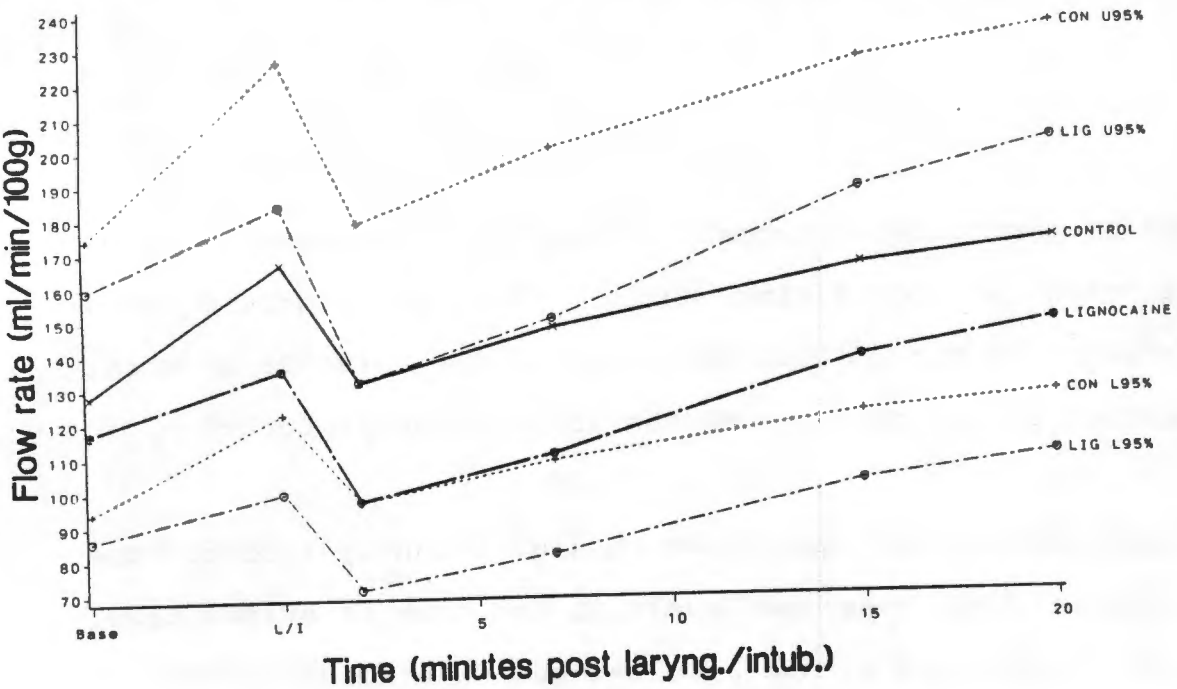
Laryngoscopy/intubation was shown to significantly increase the blood flow rate above baseline levels at the time of stimulation, and this was associated with a simultaneous significant reduction in tissue resistance. The increased flow rate was short lived, and a compensatory increase in tissue resistance was noted at 2 minutes post stimulus, effectively countering the surge.

In the cerebral grey matter the drug did not appear to significantly alter the effects of the stimulus on the brain blood flow or tissue resistance, and what changes there were may be related to differences in blood pressure, anaesthetic and arterial blood gas tensions.

4.2.2.2 Cerebellum

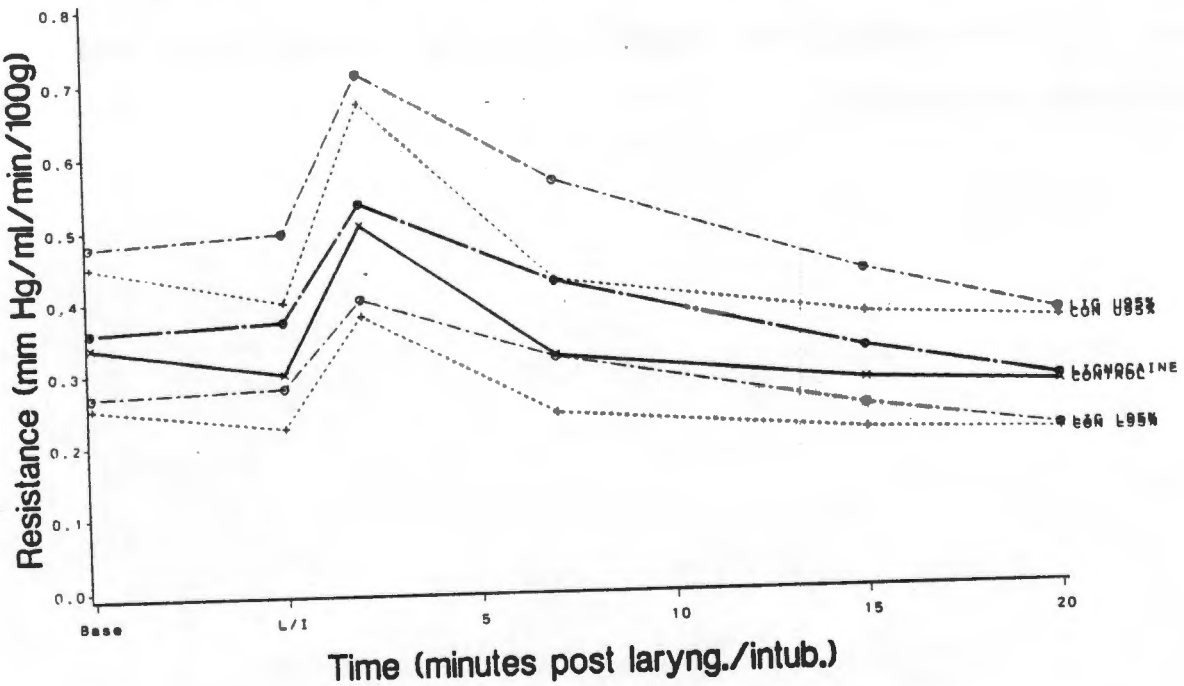
Cerebellum - regional blood flow rate

Geometric means and their 95% confidence intervals.



Cerebellum Resistance.

Geometric means and their 95% confidence intervals.



Blood flow rate in the cerebellum was significantly lower in the Lignocaine group over the whole experimental period ($p = 0.0187$). In the pairwise comparison blood flow rate was not significantly altered from the baseline level at any of the timepoints. If the decrease in blood flow following laryngoscopy/intubation is analysed however, there is a statistically significant reduction in flow within 2 minutes, regardless of the treatment group ($p = 0.047$). This confirms the presence of the same rapid regulation of blood flow at a tissue level seen in the other brain regions.

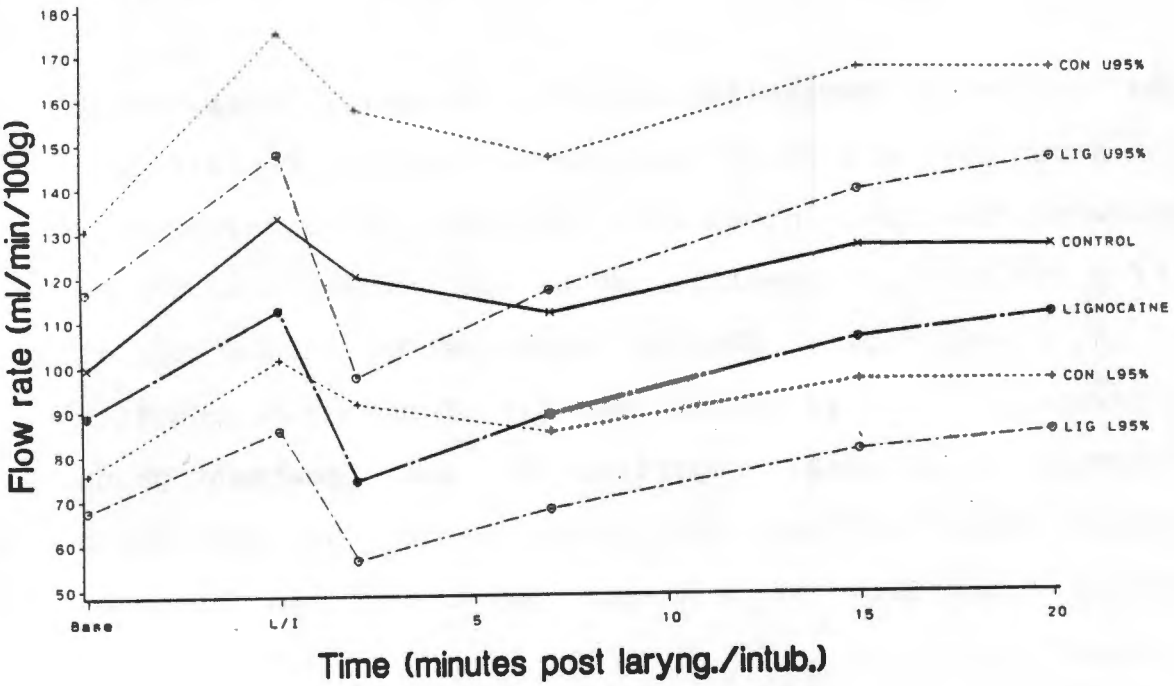
There were no other significant blood flow changes either within, or between the two groups, in the cerebellum. The data is displayed graphically on the opposite page.

There was no difference in the degree of tissue resistance seen in the two groups over the whole time period ($p = 0.094$). Nor were there any differences in tissue resistance, either within or between the two groups, when the data was analysed at the individual time points.

Pairwise comparison, however, showed that regardless of treatment group, the tissue resistance at 2 minutes post laryngoscopy/intubation was significantly elevated (>50%) above all of the other measurements ($p < 0.01$). This finding is complementary to that of the reduced blood flow rate.

Cerebral White Matter - regional blood flow rate

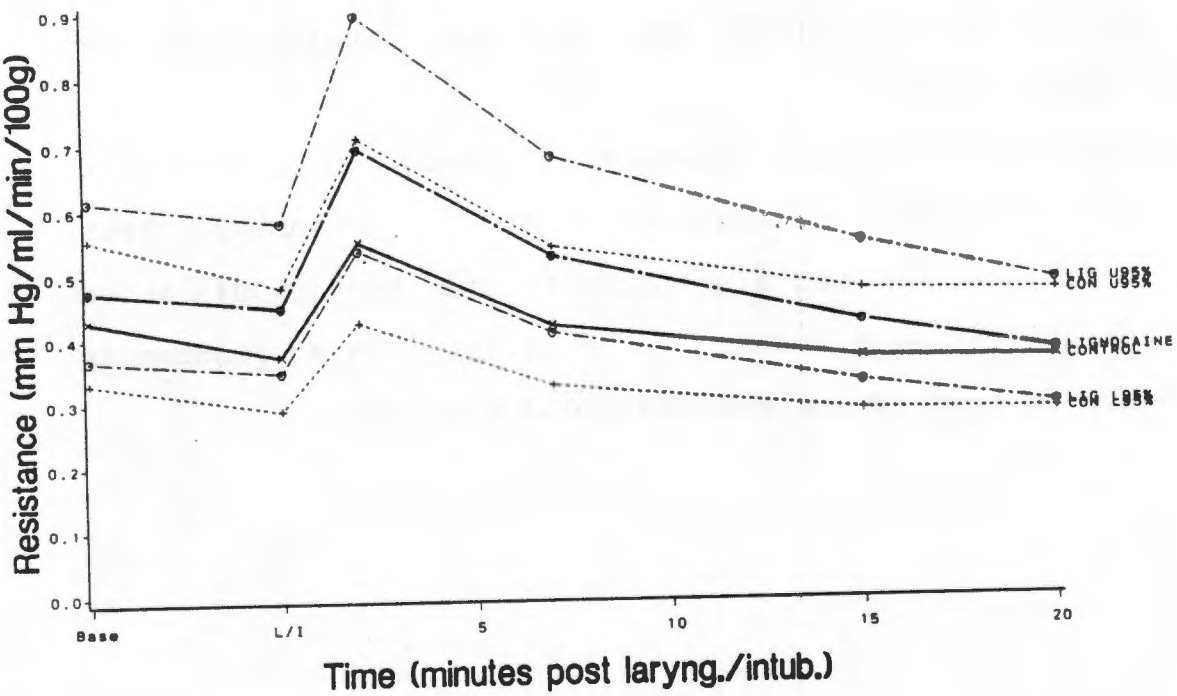
Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Cerebral White Resistance.

Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Blood flow was raised in both groups at the end of the experiment, again suggesting a possible vasodilator effect of the raised PaCO2 and prolonged anaesthetic in this brain region.

4.2.2.3 Cerebral white

Analysis of the effect of the lignocaine over the whole study period showed a highly significant treatment effect ($p = 0.0053$) in the cerebral white matter. The blood flow rate in the Lignocaine group was significantly lower than in the Control group. The data is displayed graphically on the opposite page.
Pairwise comparison showed that the blood flow rate at the time of laryngoscopy/intubation was significantly raised above the baseline ($p = 0.0384$) and the 2 minute ($p = 0.0407$) levels regardless of the treatment group. In addition, when the blood flow rates at the individual timepoints were compared, there was a significantly lower flow rate in the Lignocaine group at the 2 minute measurement ($p = 0.0086$).
Within the two groups there were no changes in flow rate from the baseline level that achieved statistical significance.

Tissue resistance in the Lignocaine group was significantly higher than in the Control group when compared over the total study period. Despite this, at no individual timepoints were there any significant differences in tissue resistance between the two groups and the shape of the two curves was very similar.

Tissue resistance did not change significantly in either group during laryngoscopy/intubation, and the increased cerebral white matter blood flow was presumably due to the increasing blood pressure at this time.

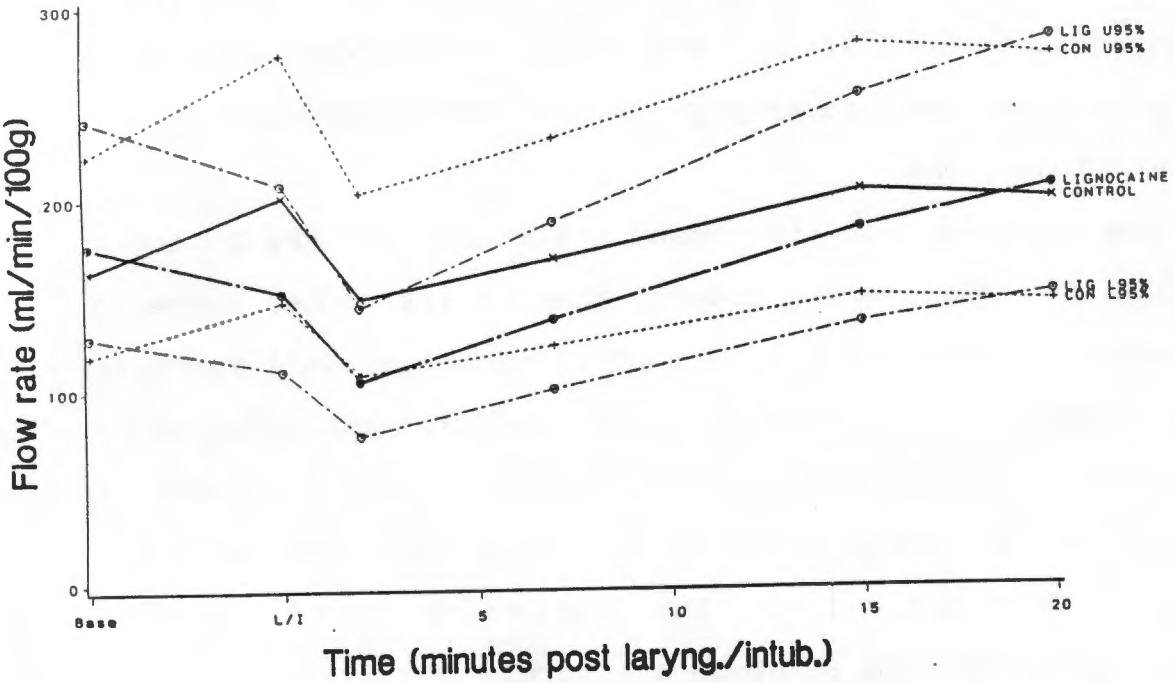
Pairwise comparison showed the tissue resistance at the 2 minute timepoint to be significantly greater than at all other times in the experiment, suggesting a powerful autoregulatory response that reset itself within 7 minutes, and which was not affected by the lignocaine. Cerebral white matter blood flow decreased in response to this increased resistance, with a much more rapid and pronounced effect noticed in the Lignocaine group, perhaps reflecting the difference in blood pressure.

Because of the large variances, the seemingly large fluctuations in tissue resistance within the two groups did not achieve statistical significance.

There appeared to be less of an anaesthetic and $PaCO_2$ vasodilator effect noted in this brain region as compared to the cerebral grey matter, with the 20 minute blood flow rates being insignificantly raised above the baseline levels.

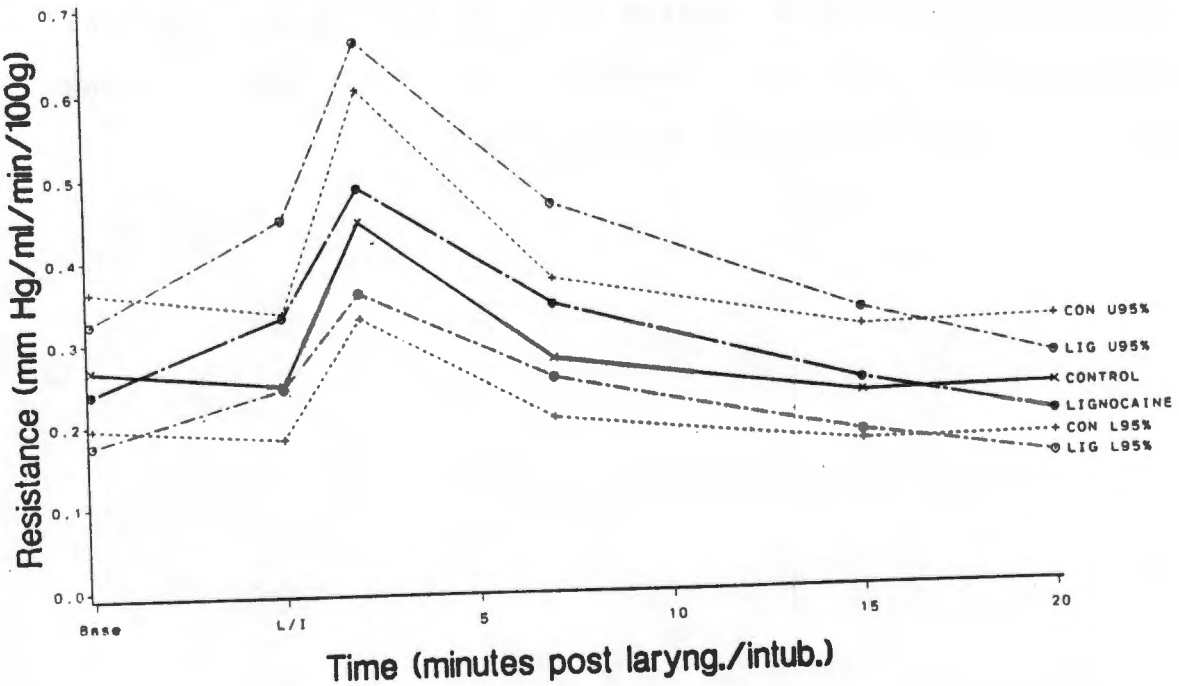
Thalamus - regional blood flow rate

Geometric means and their 95% confidence intervals.



Thalamus Resistance.

Geometric means and their 95% confidence intervals.



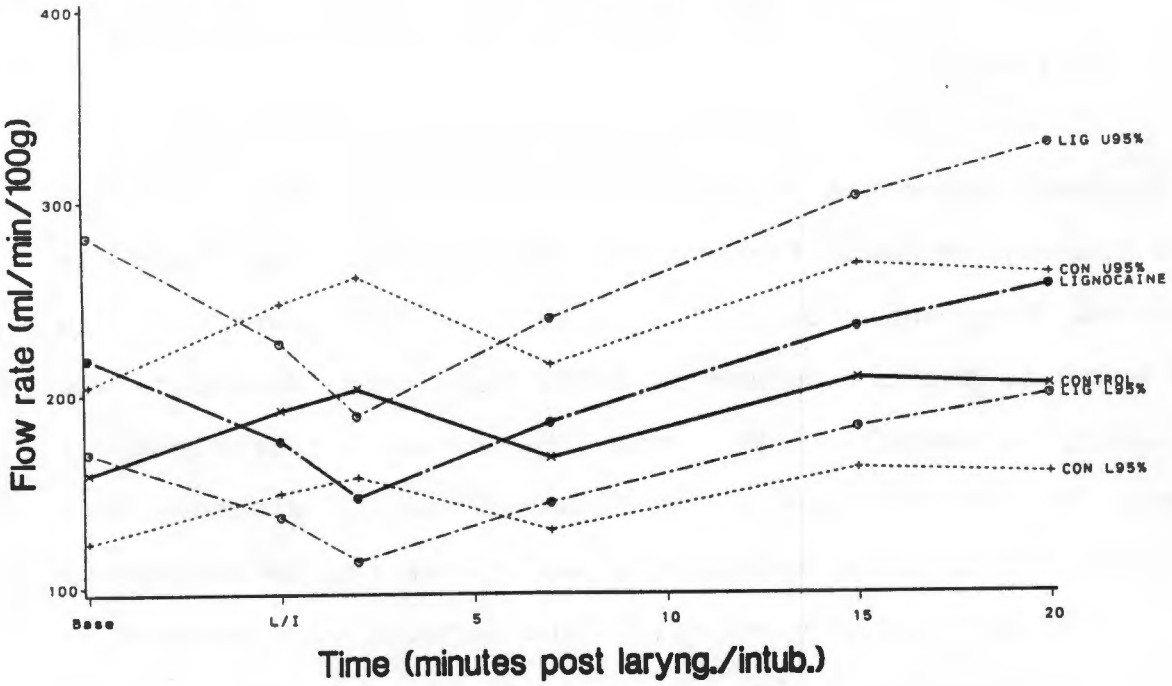
4.2.2.4 Thalamus

In the thalamus there was no demonstable effect of the lignocaine when the changes in blood flow rate of both groups over the whole study period were analysed ($p = 0.1161$). Pairwise analysis did however indicate two timepoints at which the blood flow rate was significantly elevated above the baseline, regardless of treatment,i.e. 15 minutes ($p = 0.0045$) and 20 minutes ($p = 0.0026$). This finding is important since it may indicate a higher sensitivity of the thalamic region to the effects of hypercarbia. There were no significant differences in blood flow rate between the two groups at any of the timepoints, but the pattern of blunted stimulus effect at the laryngoscopy/intubation estimation in the Lignocaine group, was maintained ($p = 0.1675$). In the Control group there were no changes in blood flow rate that achieved significance at a $p = 0.01$ level, but in the Lignocaine group the 2 minute blood flow rate was significantly lower than the baseline level ($p = 0.01$). The data is displayed graphically on the opposite page.

There was no overall difference in tissue resistance between the two groups ($p = 0.4290$). In the pairwise comparison the tissue resistance at the 2 minute timepoint was significantly higher than at all other timepoints regardless of treatment group ($p < 0.0041$). The two groups correlated well at the different timepoints and there were no significant differences. Within both

Caudate Nucleus - regional blood flow rate

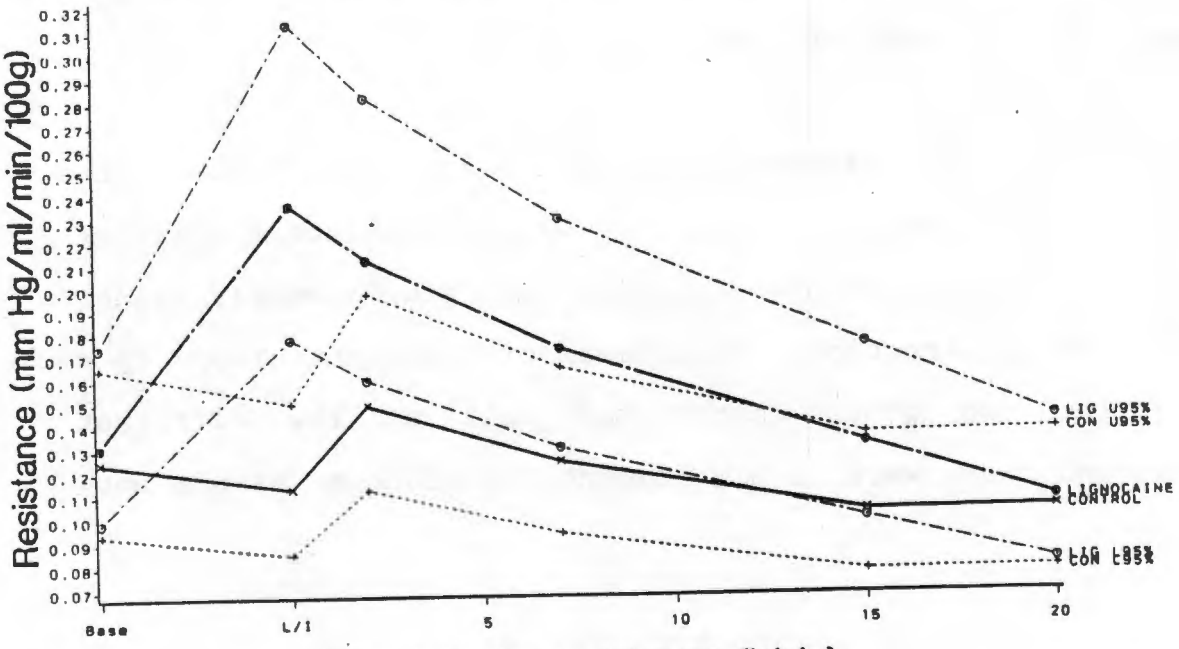
Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Caudate Nucleus Resistance.

Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

the Control ($p = 0.01$) and the Lignocaine ($p = 0.0008$) groups however, it was noted that the tissue resistance at the 2 minute timepoint was significantly higher than the baseline value.

4.2.2.5 Caudate Nucleus

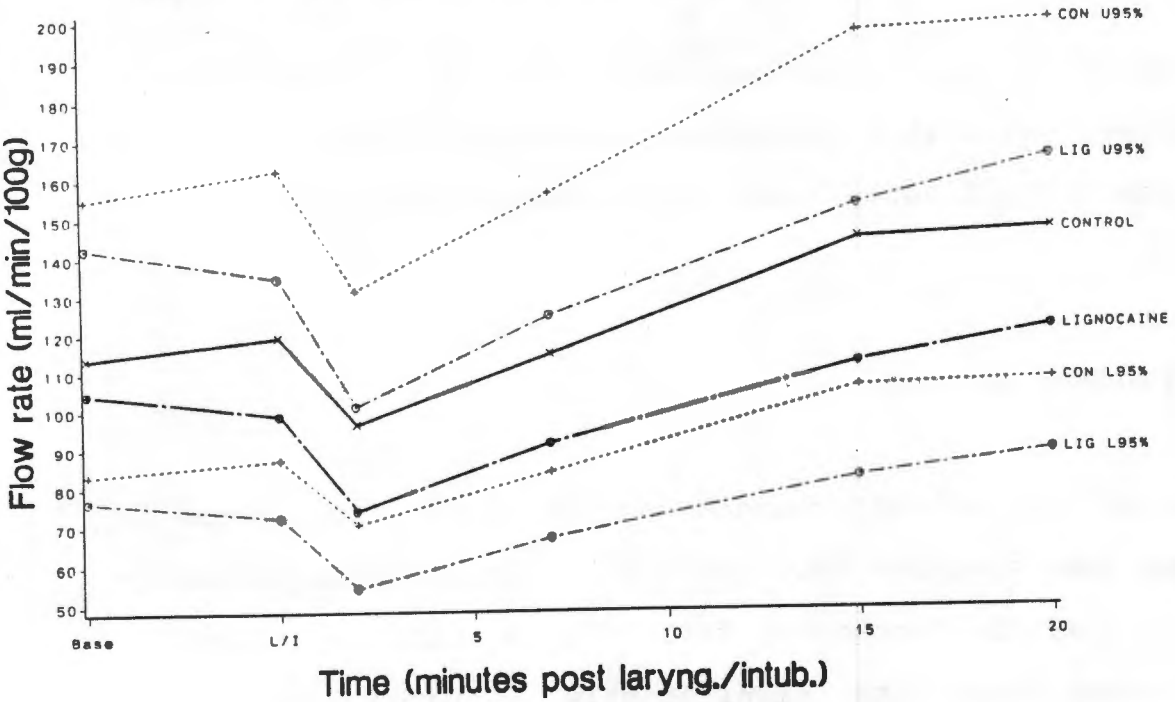
There were no significant changes in the blood flow rates in either group when analysed over the entire experimental period ($p = 0.3883$). Pairwise comparison failed to identify any timepoint that was associated with significantly different blood flow rates. Although none of the blood flow differences seen in this brain region were significant when compared for treatment effect at the individual timepoints, the pattern of blood flow is interesting since there was a 29% decrease in flow rate at 2 minutes in the Lignocaine group which approaches significance ($p = 0.0244$). This sudden decrease was not noted in the Control group in which there were no significant changes following the stimulus.

Data is displayed graphically on the opposite page.

The tissue resistance in the Lignocaine group was significantly lower than in the Control group when the whole experimental period was analysed ($p = 0.0004$). Pairwise comparison showed tissue resistance at the 2 minute timepoint to be significantly higher than at the baseline estimation ($p = 0.0141$), the 15 minute ($p = 0.0023$) and the 20 minute ($p = 0.0003$) timepoints. During the stimulus the Lignocaine group showed a significantly

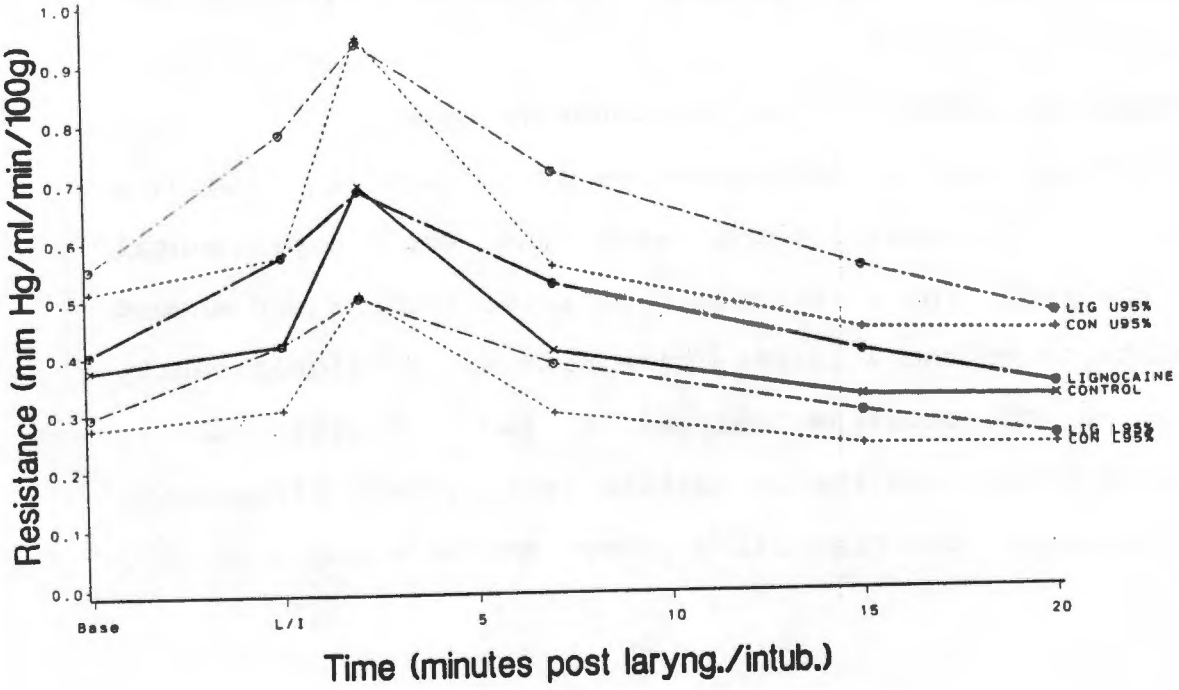
Hippocampus - regional blood flow rate

Geometric means and their 95% confidence intervals.



Hippocampus Resistance

Geometric means and their 95% confidence intervals.



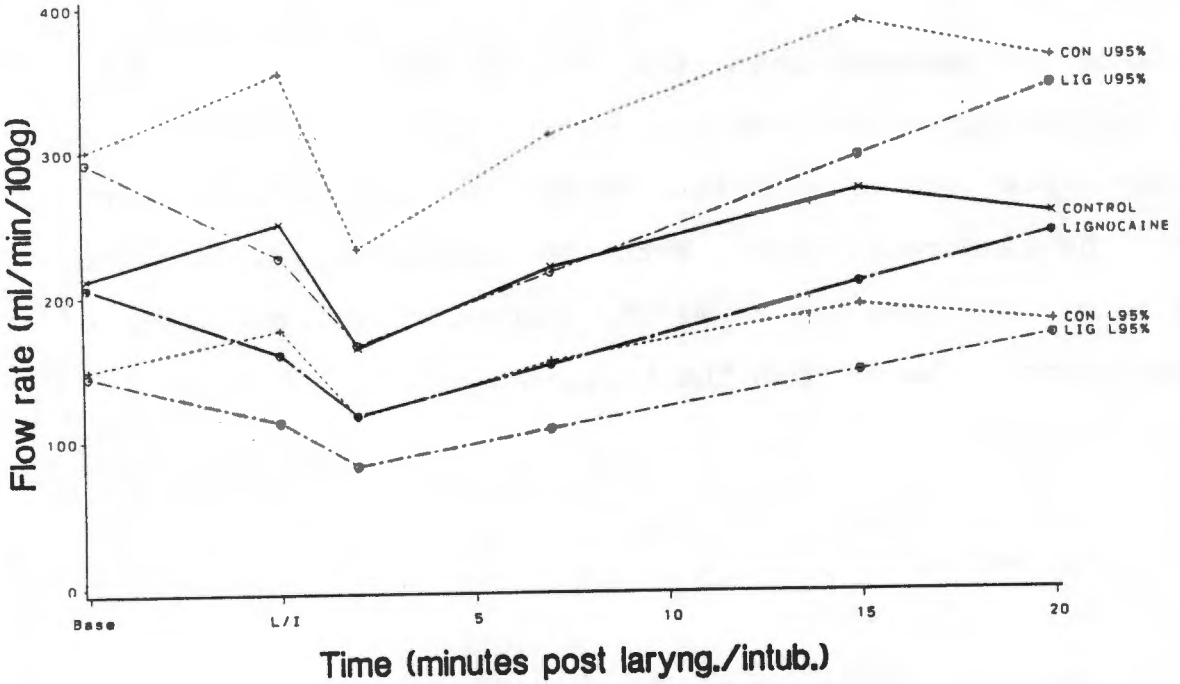
higher tissue resistance than the Control group ($p = 0.0002$), possibly indicating a more reactive vasculature. Within the two groups there were no significant changes in the Control group, but within the Lignocaine group both the laryngoscopy/intubation value ($p = 0.0024$) and the 2 minute timepoint ($p = 0.01$) value were significantly higher than the baseline.

4.2.2.6 Hippocampus

In the hippocampus there were no statistically significant decreases from the baseline blood flow rates within either of the two groups when compared for individual values at the various timepoints. The same pattern as seen in other areas was noted, viz. a blunted response during the stimulus in the Lignocaine group, but this did not achieve significance ($p = 0.0965$).
Data is graphed on the opposite page.
Overall, there was a significantly decreased the blood flow rate in the Lignocaine group over the experimental period ($p = 0.0228$), but because of the similar curve shapes of the two groups this difference may represent a resetting of the baseline flow rate due to a lower perfusion pressure, rather than a lignocaine effect at the hippocampus level.

Midbrain - regional blood flow rate

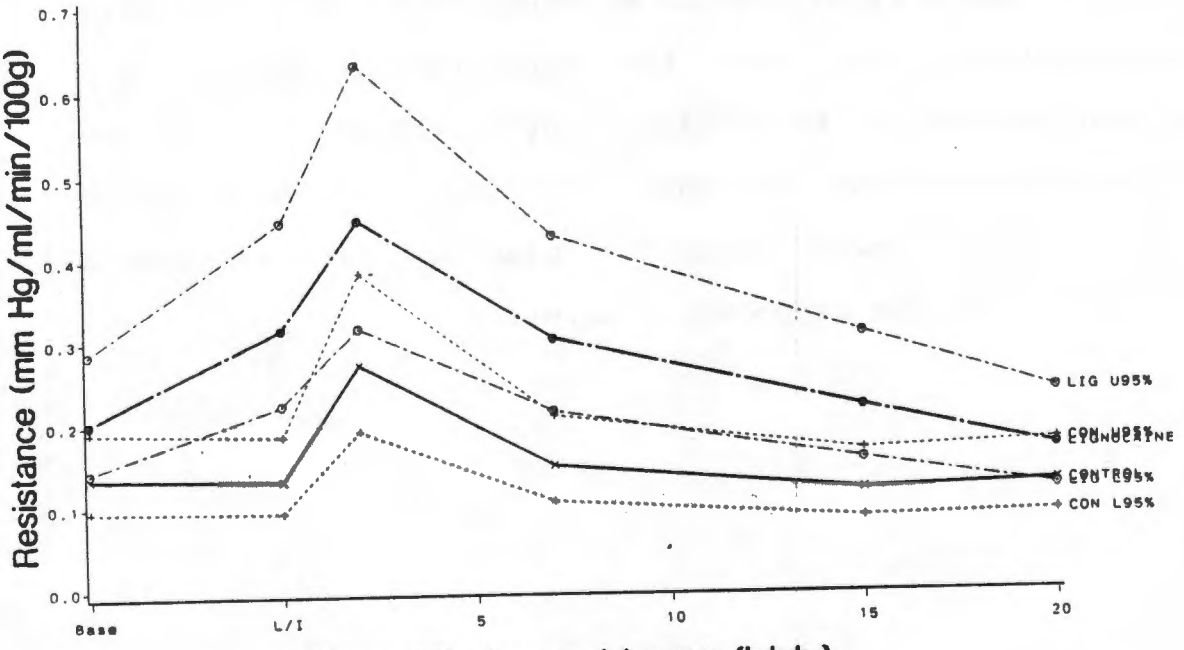
Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Midbrain Resistance.

Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Blood flow rate in both groups was significantly lower at 2 minutes post stimulus when compared with the 15 minute timepoint ($p = 0.0073$) and the 20 minute timepoint ($p = 0.0040$), indicating again that the compensatory autoregulation was not inhibited by the lignocaine.

The tissue resistance in the hippocampus was similar in the two groups when compared over the entire study period. Pairwise comparison showed that at the 2 minute post laryngoscopy/ intubation timepoint the tissue resistance was significantly elevated regardless of treatment ($p < 0.02$). Within the Control group the 2 minute resistance was significanlty elevated above the baseline level ($p = 0.0039$), as was the case in the Lignocaine group ($p = 0.01$).

4.2.2.7 Midbrain

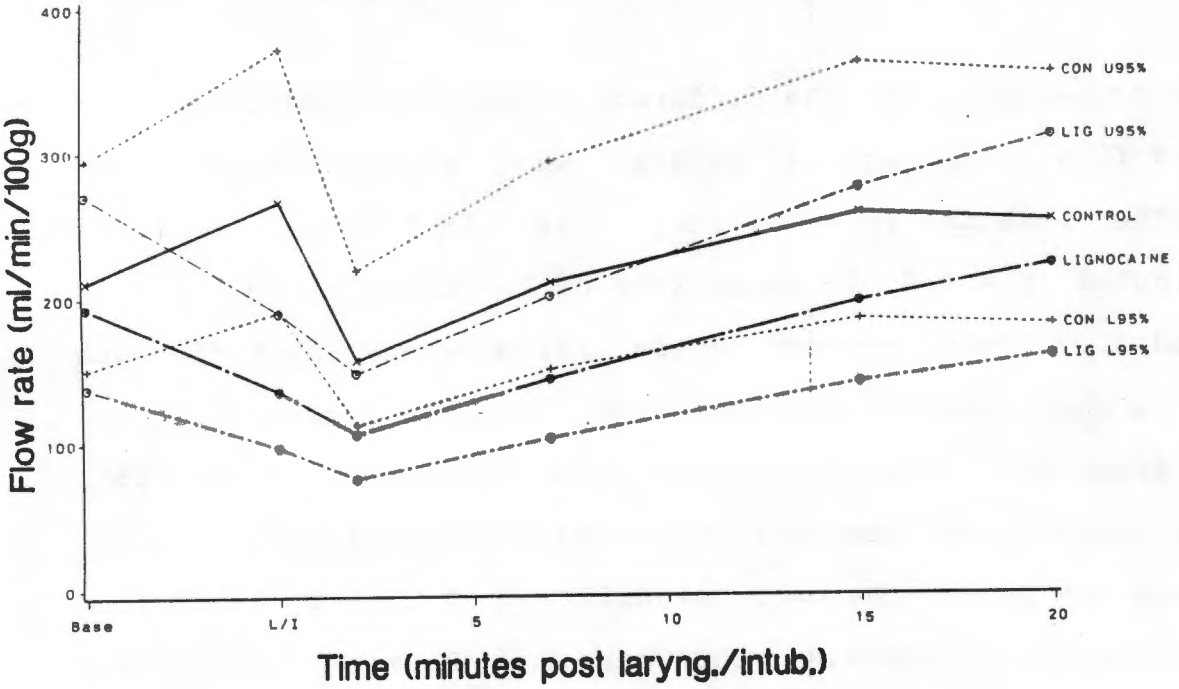
In the midbrain there was a significant lignocaine effect noted when the blood flow rates over the entire study period were analysed ($p = 0.0112$), with the treated animals showing a significantly lower blood flow rate. The data is displayed graphically on the opposite page.

Pairwise comparison at the different timepoints indicated that the blood flow rate at 2 minutes was significantly lower than at the baseline ($p = 0.0124$), 15 minutes ($p = 0.0018$) or 20 minutes ($p = 0.0011$) regardless of treatment group.
The blood flow rate pattern during laryngoscopy/intubation was similar to that seen in the brainstem, with an increase in the Control group and a decrease in the Lignocaine group ($p = 0.05$). This was the only difference that tended to significance.
Within the two groups the only significant change in blood flow rate was in the Lignocaine group where the 2 minute estimation was significantly lower than the baseline (43%); this reduction was not seen in the Control group.

Tissue resistance in the Lignocaine group was significantly higher than in the Control animals when analysed over the whole 20 minutes of the study period ($p = 0.0001$).
Pairwise comparison showed that irrespective of the treatment group the tissue resistance was significantly higher at 2 minutes post stimulus than at any other timepoint ($p < 0.0026$).
When the tissue resistances at the individual timepoints were assessed, there was significantly greater resistance noted in the Lignocaine group than in the Control group during laryngoscopy/ intubation ($p = 0.0003$), at 7 minutes ($p = 0.0023$) and at 15 minutes ($p = 0.0114$) post stimulus. This suggests a more rapid

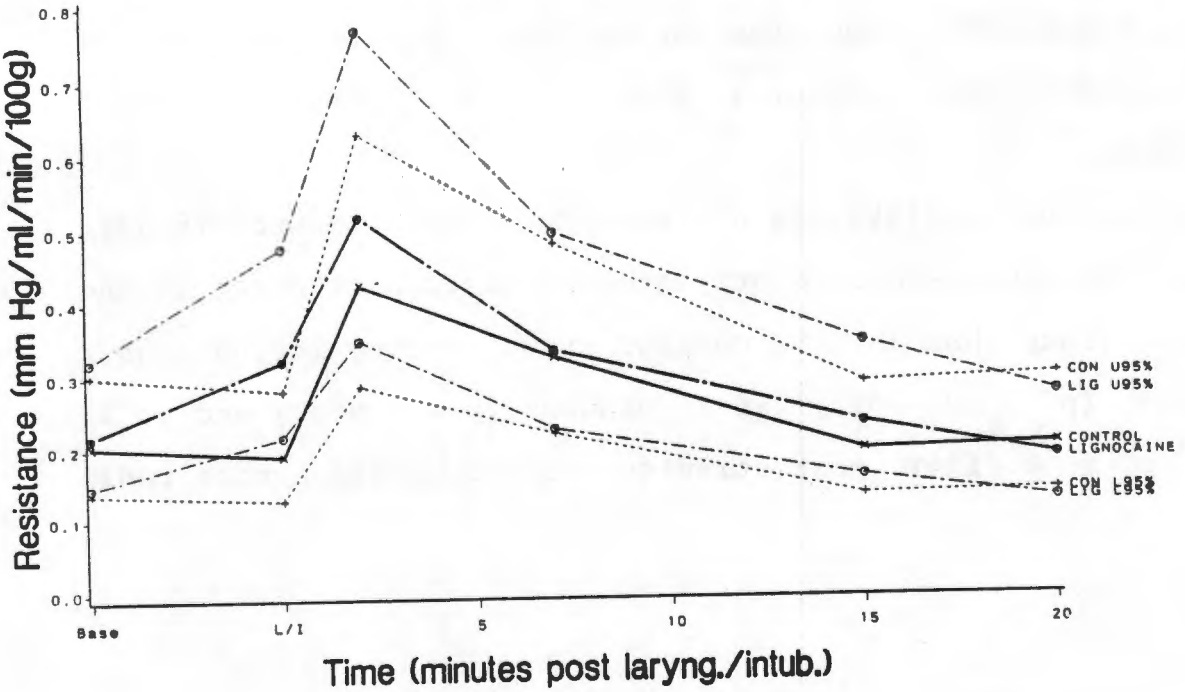
Pons - regional blood flow rate

Geometric means and their 95% confidence intervals.



Pons Resistance.

Geometric means and their 95% confidence intervals.



and prolonged autoregulatory response to laryngoscopy/intubation in the Lignocaine group, again highlighting the potential protective effect of the drug against such blood pressure surges. Comparison of tissue resistance within each group showed that resistance was significantly increased from the baseline level in both groups only at the 2 minute timepoint ($p < 0.0032$).

4.2.2.8 Pons

The different blood flow pattern during laryngoscopy/intubation was reiterated in the pons, with a 30% decrease in blood flow in the Lignocaine group, and a 22% increase in the Control group. The data is displayed graphically on the opposite page.
The Lignocaine group showed a significantly reduced flow rate ($p = 0.001$) over the total experimental period when compared with the Control group, confirming a significant treatment effect. In addition, at the laryngoscopy/intubation timepoint the flow rate in the Lignocaine group was significantly less than in the Control group ($p = 0.0049$). Thus, during laryngoscopy/intubation lignocaine significantly reduced the stimulus effect in the pons.

In the pairwise comparison the blood flow rate at 2 minutes post laryngoscopy/intubation was significantly less than at all of the other timepoints except for the 7 minute post laryngoscopy/

intubation timepoint, regardless of treatment group. This implies that there was rapid compensatory autoregulation which was unaffected by the lignocaine.

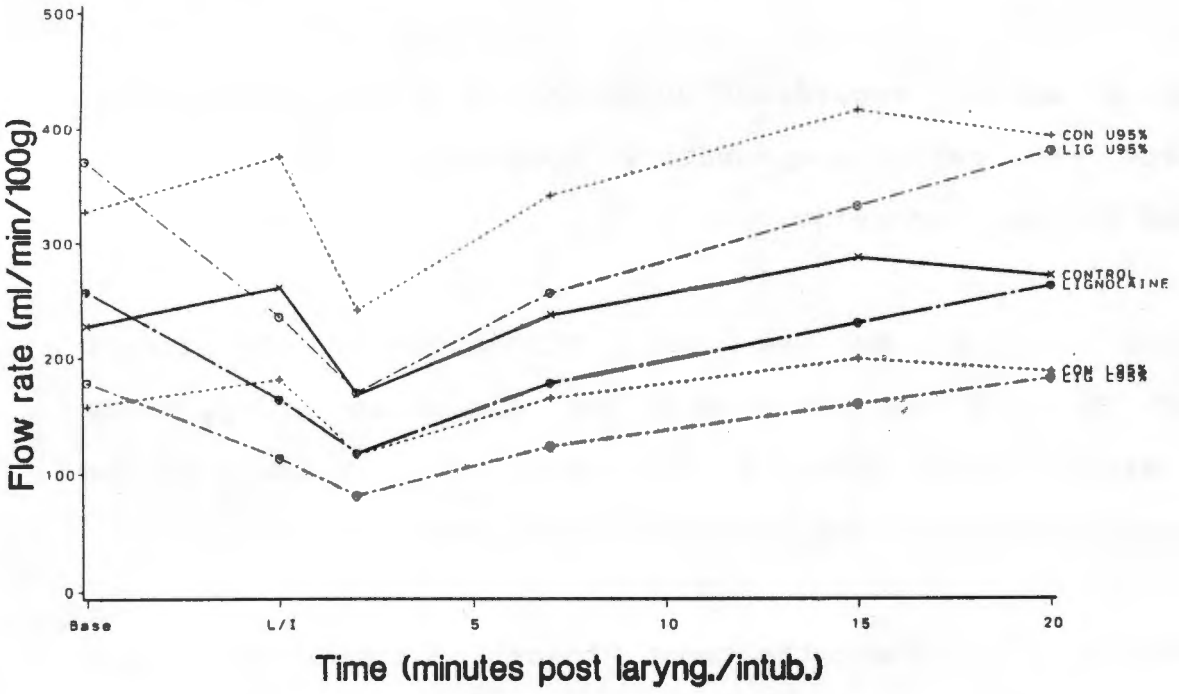
Within the two groups the only statistically significant difference in blood flow was seen in the Lignocaine group, where the 2 minute post stimulus flow rate was noted to be significantly less than the baseline value ($p = 0.0073$).

There was no significant treatment effect on tissue resistance when the two groups were compared over the entire experimental period ($p = 0.1599$). This finding suggests that any flow rate differences between the two groups is probably on the basis of perfusion pressure differences.

In the pairwise comparison tissue resistance at the 2 minute timepoint was significantly higher than at all other times except for the 7 minute timepoint ($p < 0.008$) indicating a fairly rapid autoregulation (within 7 minutes) for the increased flow rate induced by the stimulus . There were no significant differences between the two groups when pons tissue resistance was compared at any of the timepoints, reiterating the close correlation between the two curves and indicating an apparent lack of drug effect at a tissue level. Lignocaine treatment did however appear to increase the tissue resistance at the time of laryngoscopy/intubation, as opposed to the decrease noted in the

Medulla - regional blood flow rate

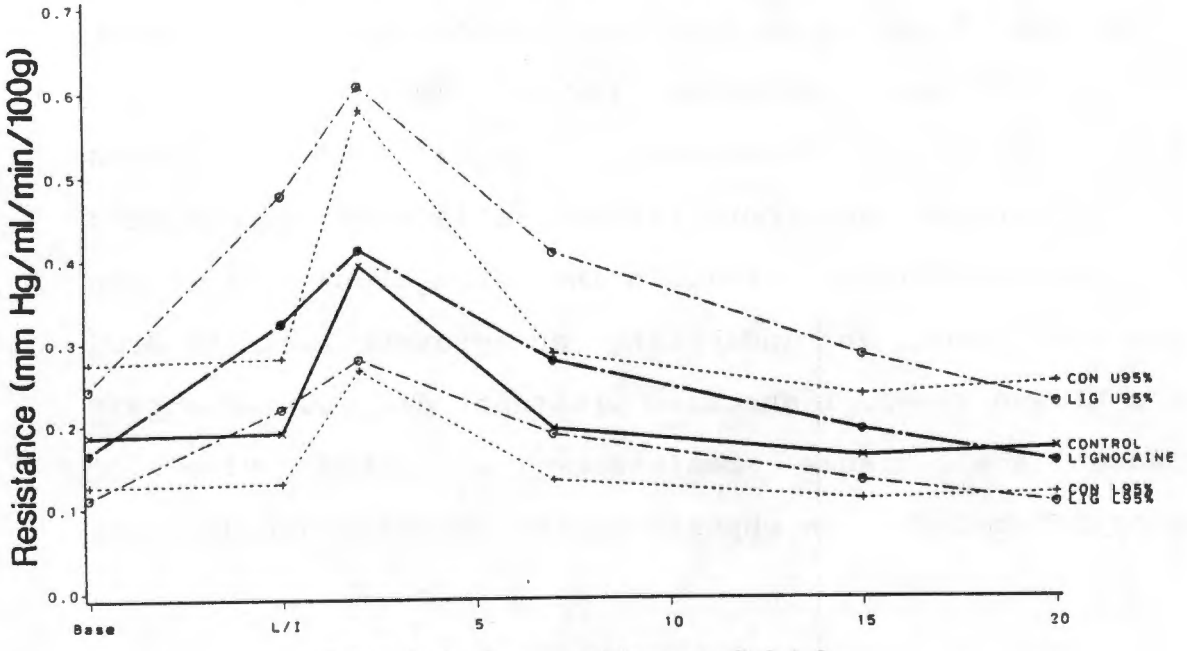
Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Medulla Resistance.

Geometric means and their 95% confidence intervals.



Time (minutes post laryng./intub.)

Control group, but this difference ($p = 0.041$) did not achieve significance at a $p = 0.01$ level, possibily due to the small number of observations.

Within the two groups there was a significantly higher tissue resistance at the 2 minute post stimulus timepoint, with the increase being more significant in the Lignocaine group ($p = 0.0012$) than in the Control group ($p = 0.0057$).

4.2.2.9 Medulla

The blood flow rate in the medulla was significantly lower in the Lignocaine group over the total study period when compared with the Control group ($p = 0.0493$). The flow rate at the 2 minute timepoint was significantly lower than that seen at the baseline ($p = 0.0021$), 15 minute ($p = 0.0009$) or 20 minute ($p = 0.0006$) estimations, regardless of the treatment group. There were no significant differences between the flow rates in the Lignocaine or Control groups at any of the timepoints, despite the more pronounced reduction in flow during laryngoscopy/intubation in the Lignocaine group (33%). The data is displayed graphically on the opposite page.

Within the groups, analysis showed a significantly reduced blood flow rate in the Lignocaine group at the 2 minute timepoint (54%) when compared with the baseline ($p = 0.0017$). This degree of reduction was not seen in the Control group (34%).

There was no difference in the medulla tissue resistance between the two groups over the course of the whole experimental period ($p = 0.164$). Pairwise comparison revealed that the 2 minute post laryngoscopy/intubation tissue resistance was significantly higher than at all other measured times ($p < 0.0089$). When the tissue resistance between the two groups was compared at the individual timepoints, the Lignocaine group showed a higher tissue resistance (161%) than the Control group (100%) at the 2 minute timepoint ($p = 0.0386$).
Comparison of values within the groups revealed significantly elevated tissue resistance in the Control group at the 2 minute timepoint ($p = 0.0044$), and in the Lignocaine group at both the laryngoscopy/intubation ($p = 0.0094$) and the 2 minute post stimulus ($p = 0.0006$) timepoints.

Blood flow rates in the medulla were higher throughout the experiment than those seen in the cerebral grey and white matter, suggesting that either the medulla is more sensitive to a raised PaCO2, or less sensitive to a raised PaO2, than the cerebrum. The cerebral grey blood flow increased equally in the two groups at the end of the experimental period when there was a marked increase in the PaCO2. This tends to support the theory that the cerebral grey is more resistant to raised PaCO2 levels than the other tissue regions, which were already nearly maximally vasodilated at a lower PaCO2, and therefore did not show any significant changes in the latter stages of the experiment. It

also suggests that the stimulus of the laryngoscopy/intubation is capable of overcoming this vasodilatation.

4.2.2.10 Upper cervical

The highest blood flow rates recorded in this study were noted in the upper cervical area in the Control group. A mean baseline flow rate in excess of 340 ml/min/100g was seen. Overall, there was a highly significant treatment effect, with the flow rates in the Lignocaine group lower than those in the Control group over the total experiment time ($p = 0.0001$). The curve shape was similar in both groups, and the likelihood that the observed difference was on the basis of reduced perfusion pressure is high. Graphic representation of the data is shown on the opposite page.

Pairwise comparison showed the 2 minute post stimulus flow rate to be significantly lower than those seen at baseline ($p = 0.0077$), 15 minute ($p = 0.0094$) and 20 minute ($p = 0.0078$) timepoints.

Blood flow rates in the Lignocaine group were significantly lower than those seen in the Control group at the time of laryngoscopy/intubation ($p = 0.0001$), and at 2 minutes ($p = 0.0001$), 7 minutes ($p = 0.0030$) and 15 minutes ($p = 0.0003$) post stimulus.

Within the Control group there were no individual values at any of the timepoints that were significantly different from the baseline. In the Lignocaine group however blood flow was significantly reduced (51%) at 2 minutes ($p = 0.0008$).
Tissue resistance in the Lignocaine group was shown to be significantly greater than in the Control group during the experimental period. In addition, pairwise comparison demonstrated that regardless of treatment, the tissue resistance in the upper cervical region at 2 minutes post stimulus was significantly greater than at any other time during the study ($p < 0.0045$).

There were no significant differences in resistance between the two groups at any of the timepoints, but comparisons within both the Lignocaine ($p = 0.0003$) and the Control groups ($p = 0.002$) showed that the tissue resistance at 2 minutes was significantly greater than at their respective baseline values.

4.2.3 Curve correlation

4.2.3.1 Blood flow rates

TABLE P3-14: Correlation of the blood flow rate curve shape between the Control and Lignocaine groups in the different brain regions over the whole experimental time period.

BRAIN REGION	r VALUE
LOW FLOW REGIONS	
CEREBRAL GREY	0.786
CEREBRAL WHITE	0.097
CEREBELLUM	0.782
HIGH FLOW REGIONS	
CAUDATE NUCLEUS	0.135
HIPPOCAMPUS	0.451
MIDBRAIN	0.542
PONS	0.549
MEDULLA	0.737
THALAMUS	0.755
UPPER CERVICAL	0.800

4.2.3.2 Tissue resistances

TABLE P3-15: Correlation of the tissue resistance curve shape between the Control and Lignocaine groups in the different brain regions over the whole experimental time period.

BRAIN REGION	r VALUE
LOW FLOW REGIONS	
CEREBRAL GREY	0.912
CEREBELLUM	0.726
CEREBRAL WHITE	0.423

HIGH FLOW REGIONS	
HIPPOCAMPUS	0.470
CAUDATE NUCLEUS	0.597
MIDBRAIN	0.668
MEDULLA	0.672
UPPER CERVICAL	0.695
PONS	0.764
THALAMUS	0.804

The correlation coefficients in the above two tables are provided for completeness sake only. There are too few points on the curves to allow adequate assessment of curve correlation over the critical first 7 minutes of response, and for this reason analysis has been over the full 20 minutes. This has decreased the sensitivity of the analysis in most cases. The trends are

however interesting, and in the discussion below these will be covered briefly.

4.3 Summary of Results

4.3.1 Cardiovascular

The cardiovascular findings in phase 3 were essentially confirmatory of those seen in the other 2 phases of the study. Laryngoscopy and intubation was shown to be a potent stimulus capable of significantly elevating blood pressure (+/- 50%), heart rate (+/- 27%) and cardiac output (+/- 140%) within 2 minutes of initiatation. There was a definite delay in the haemodynamic response, with the greatest rate of change being noted after the animals had already been subjected to 30 seconds of laryngoscopy. This lag period may explain why some investigators claim that there is a minimal response to laryngoscopy in neonates; their monitoring intervals may well be set too early, with the result that the peak changes are not recorded.

The peak blood pressure response in this study occurred at 4 minutes, and in the untreated state approximately 12 minutes was required for the pressure to return to the baseline value, indicating the prolonged nature of the response. Lignocaine

significantly reduced the peak pressure seen after laryngoscopy and intubation and shortened the time of exposure to the elevated blood pressure levels.

Thus lignocaine appears to exert a protective effect in terms of limiting both the degree of the blood pressure increase and the duration of exposure to the elevated pressures. An interesting aspect of the blood pressure response was noted after 30 seconds of laryngoscopy where both groups showed essentially identical pressure increases. The lignocaine did not appear to have had any effect at this time, but in the ensuing minutes there was a significant difference in pressure between the two groups that has been ascribed to a lignocaine effect. Whether the lignocaine specifically reduced the extent of the response to intubation alone, or whether the lignocaine had its main effect on the delayed reaction to the combination of laryngoscopy and intubation, cannot not be explained on the basis of these results. In a clinical sense this distinction is relatively unimportant, since laryngoscopy is rarely performed without some aspect of irritation to the supraglottic larynx.

Heart rate was also significantly increased by the laryngoscopy/ intubation with little evidence of the previously reported vagally induced bradycardia. Lignocaine again appeared to demonstrate a protective effect by significantly reducing the extent and duration of the heart rate increase.

Cardiac output increased significantly following laryngoscopy/ intubation, and the surge in output showed a similar pattern to that seen with the blood pressure. The only significant

lignocaine effect appeared to be a more pronounced increase in cardiac output during the laryngoscopy/intubation timepoint. Since this was associated with similar blood pressure levels between the two groups, it substantiates the theory that the lignocaine caused peripheral vasodilatation.

4.3.2 Cerebrovascular

There were a number of important findings in the analysis of the brain blood flow rates in the different areas studied. Some of these confirmed previous findings, but more importantly two distinct patterns of brain blood flow response during laryngoscopy/intubation were noted, and a potential brain stem protective effect of lignocaine was shown.

Hypercarbia was demonstrated to have a potent effect on brain blood flow, increasing the baseline flow rate significantly, when compared to flow rates seen in normocarbic animals (Wagerle et al, 1986). In addition there was a differential increase with much higher flow rates being noted in the brain stem as compared to higher regions. This is significant in the light of the previously mentioned phase 1 findings, where bleeding was shown to have occurred predominantly in the basal region. Hypercarbic animals may thus be predisposed to fluid and protein extravasation, and under extreme circumstances, haemorrhage in

the brain stem, by virtue of this exposure to prolonged periods of very high blood flow, (and by extrapolation, high perfusion pressure).

Because of this differentially increased blood flow it was possible to separate the brain into high flow (> 150 ml/min/100g) and low flow regions during hypercarbia. The high flow areas included the thalamus, caudate nucleus, hippocampus, midbrain, pons, medulla and upper cervical regions. The low flow brain areas were the cerebrum (white and grey matter) and the cerebellum.

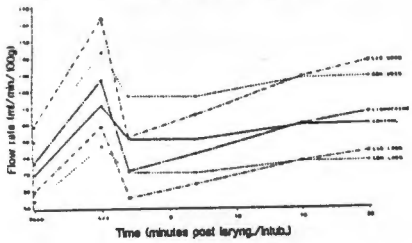
Baseline blood flow rates in some of the high flow brain stem regions far exceeded peak flow rates noted during the post laryngoscopy surge in the low flow cerebrum and cerebellum tissue. The highest baseline flow rates were seen in the more distal brain regions, and there appeared to be a gradient of regional blood flow during hypercarbia.

Flow rate in the medulla and upper cervical regions did not increase above the baseline rate at any time during the experiment (despite significant increases in blood pressure and in hypercarbia), implying that there was maximal cerebral vasodilatation present in these areas at the outset. Thus it seems likely that the upper cervical and medulla areas are more sensitive to the effects of raised carbon dioxide tension than other brain regions, and that the cerebral autoregulatory system, under the conditions pertaining at the baseline estimation, was

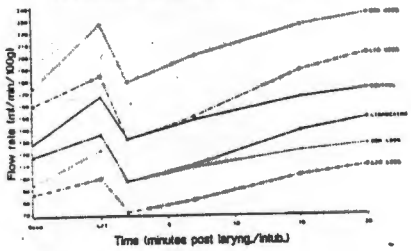
incapable of reducing the high blood flow rate. Since there was no evidence of brain bleeding other than that in the central canal in these two brain areas in the phase 1 study, it may be that the most distal regions of the brain are protected from bleeding at high flow rates. The observed haemorrhage may originate from tissue in the midbrain regions where high flow rate during hypercarbia is compounded by surges of pressure following laryngoscopy/intubation that exceed the bleeding threshold.

In all other areas of the brain, laryngoscopy and intubation was shown to induce a surge in blood flow with the maximum blood flow rate not exceeding 300 ml/min/100g. This surge in brain blood flow occurred at the time of laryngoscopy, before the blood pressure reached peak levels, and was followed almost immediately by a rapid reduction in flow rate probably on the basis of compensatory cerebral vasoconstriction. In all cases the brain blood flow rate at the time of the maximal blood pressure peak was lower than that seen during laryngoscopy. This implies that there was resetting of the cerebral autoregulation during hypercarbia, rather than the total loss of the cerebral blood flow regulation mechanism, and that in an as yet unelucidated manner, the laryngoscopy/intubation stimulated the cerebral vessels to constrict. This compensation was short lived in most regions and as the hypercarbia increased during the experiment so did the brain blood flow rates.

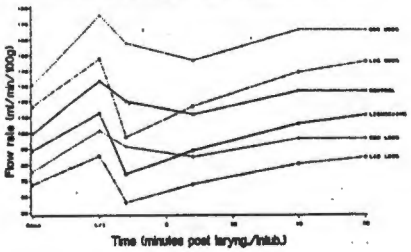
Cerebral Grey Matter - regional blood flow rate
Geometric means and their 95% confidence intervals.



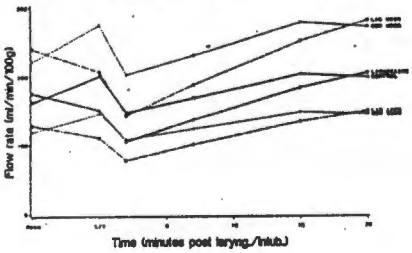
Cerebellum - regional blood flow rate
Geometric means and their 95% confidence intervals.



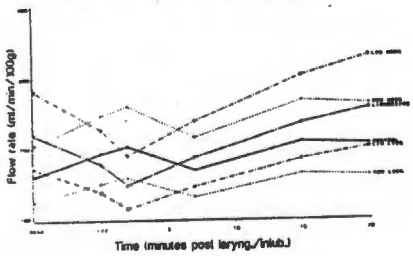
Cerebral White Matter - regional blood flow rate
Geometric means and their 95% confidence intervals.



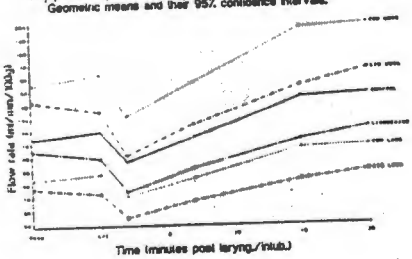
Thalamus - regional blood flow rate
Geometric means and their 95% confidence intervals.



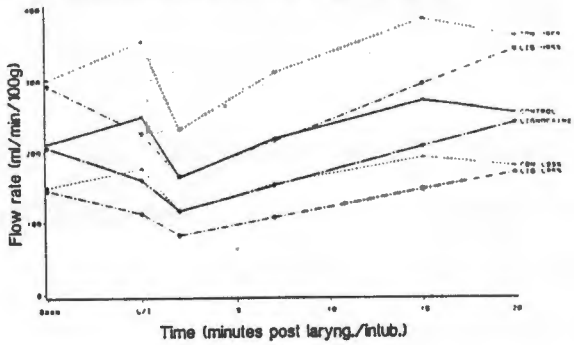
Caudate Nucleus - regional blood flow rate
Geometric means and their 95% confidence intervals.



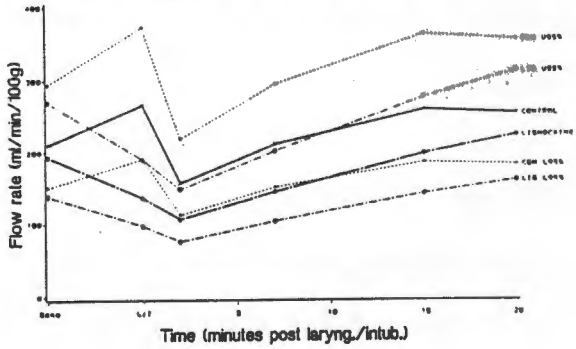
Hippocampus - regional blood flow rate
Geometric means and their 95% confidence intervals.



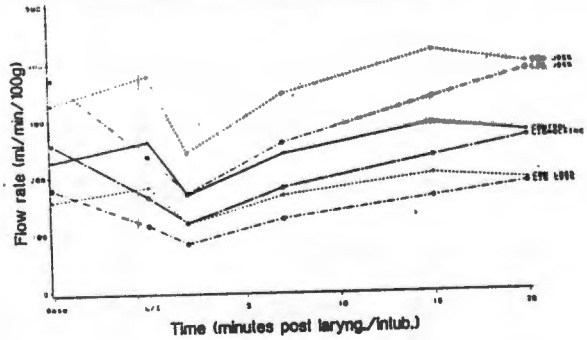
Midbrain - regional blood flow rate
Geometric means and their 95% confidence intervals.



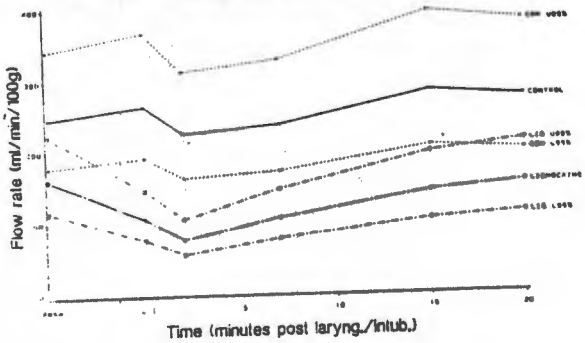
Pons - regional blood flow rate
Geometric means and their 95% confidence intervals.



Medulla - regional blood flow rate
Geometric means and their 95% confidence intervals.



Upper Cervical - regional blood flow rate
Geometric means and their 95% confidence intervals.



Two distinct patterns of blood flow were identified in response to laryngoscopy/intubation, dependant on whether or not lignocaine spray was used.

1. In the low-flow areas there was an increase in blood flow in both groups during the laryngoscopy. The lignocaine did not appear to prevent a surge in blood flow after the stimulus, but did accentuate the following compensatory vasoconstrictive response. The correlation in curve shape between the two groups was good.

2. In the high-flow areas the animals treated with lignocaine did not show the characteristic surge in brain blood flow during laryngoscopy. This blunted response was most obvious in the thalamus, midbrain, pons and medulla. Also, in these regions the 2 minute post-laryngoscopy/intubation flow rates in the Lignocaine group were disproportionately lower than those seen in the Control group, again suggesting some additive effect of the lignocaine on the compensatory vasoconstrictive response of the cerebral vessels. There was also, in most cases, a poor correlation between the two groups in terms of curve shape.

Graphs of the data are provided on the opposite page to allow comparison.

4.4 Discussion Phase 3

4.4.1 Cardiovascular

The cardiovascular findings in phase 3 were very similar to those in phase 2. In terms of the systolic pressure both the Saline/Control and the Lignocaine groups of animals in both phases had similar baseline levels, and both showed significant increases in peak mean arterial pressure following intubation. The significantly lower peak systolic pressure after laryngoscopy/intubation in the phase 3 Lignocaine group when compared to the Control group, indicates again, as seen in phase 2, the damping effect of lignocaine on the arterial pressure response.

The decreased hypertensive response following lignocaine usage in phase 3 appears to be on much the same basis as suggested in the phase 2 discussion, i.e. reduced peripheral vascular resistance, most probably related to a vasodilator effect of the drug. The figure opposite page 256 illustrates the trend in the peripheral vascular resistance showing a lower resistance in the Lignocaine group estimations throughout the experiment. The trends may explain some of the apparent group differences, but because of the small sample size and the large variances, as well as the few data points (because of the intermittant nature of the estimation method) there are no significant differences at the $p = 0.05$ level. Extrapolation of the peripheral vascular resistance (SVR) and cardiac output (CO) trend lines to points corresponding with

trend lines to points corresponding with the peak mean arterial pressure value show significant differences in the SVR and CO values between the two groups. These differences in SVR and CO correspond with the significant systolic and mean arterial pressure differences noted at the peak time point.

In phase 3 however, in addition to the peripheral vascular resistance reduction, there appears to be a difference in the cardiac output responses as well. The interpretation of the cardiac output changes in this phase is complicated by the fact that the baseline levels were different. Although this was not a statistically significant difference, the subsequent changes are confusing when one compares the percentage change with change in absolute flow rates. At the laryngoscopy/intubation timepoint the Lignocaine group had a significantly higher cardiac output than the Control group in terms of ml/min/100g units. However if one looks at the percentage change in cardiac output, the increase in the Lignocaine group was insignificantly greater than that seen in the Control group. At the 2 minute post intubation time point there was no significant difference in cardiac output values between the two groups (Table P4-13); however when the percentage change from the baseline cardiac output was calculated there was a significantly greater increase ($p<0.05$) noted in the Control group, suggesting that the lignocaine depressed the cardiac output response to the stimulus. In this analysis it has been assumed that the percentage change values offer a better indication of the actual changes, and that the apparent depressant effect of the lignocaine on the cardiac output is

real. Thus it appears that the cardiac depressant effect of lignocaine reported in adults is also seen in neonates following laryngotracheal topical application.

During laryngoscopy in phase 2 there was a significant increase ($p<0.01$) in cardiac output in both the Saline and Lignocaine groups which was not seen in phase 3 until 2 minutes later, despite there being no difference in the pattern of blood pressure changes following laryngoscopy/intubation in either phase. Although there was a significant increase in cardiac output in the phase 3 Lignocaine group during laryngoscopy/ intubation (55%), this was much less than the increase seen in the phase 2 Lignocaine group (151%) during laryngoscopy. The insignificant increase in cardiac output during laryngoscopy/ intubation in the phase 3 Control group (25%) as compared to the 130% increase in the phase 2 Saline group during laryngoscopy is also difficult to explain. It is possible that the act of intubation in some way depressed the cardiac output on a direct neural basis for as long as the irritation of the deeper laryngeal structures and vocal cords continued. The local anaesthetic blocking effect of lignocaine would attenuate the neural response to intubation and decrease the degree to which the cardiac output was depressed.

The nervous system response to stimulation of the larynx and vocal cords is graded, with different levels and types of response occurring from irritation in different areas of the respiratory tree (Tomori and Widdicombe, 1969). Thus in neonatal

piglets it seems that laryngeal irritation (laryngoscopy) stimulates a cardiac output increase, and that deeper vocal cord and tracheal stimulation leads to an attenuation of this increase. This mechanism would explain the smaller cardiac output increase seen during laryngoscopy/intubation in the phase 3 Control group. The blunting of the intensity of the intubation stimulus by laryngeal and tracheal topical anaesthesia, would lead to a less depressed cardiac output response in the phase 3 Lignocaine group. Once the vocal cord and tracheobronchial irritation was completed, the myocardial depression was arrested and the changes in the two groups then approximated each other. This mechanism would imply a rather more complex nervous system interplay than is usually considered. The organization of the autonomic nervous system has, over the last decade, been shown to be a lot more complex than previously thought. The concept of a two component autonomic nervous system fails to take into account observations that point to the existence of neuronal mechanisms that are neither adrenergic nor cholinergic (Burnstock, 1972). Neuropeptides have been shown to co-exist with "classical" neurotransmitters such as acetylcholine and noradrenaline in autonomic nerves, and in addition it has been found that one neuron may produce several peptides (Burnstock, 1972). There are several neurotransmitters found in the upper respiratory tract that may have a functional role in the mediation of the reflex response suggested above.

Cardiac output may be rapidly altered by changes in vascular beds both within and outside the pulmonary circulation (Guyton, 1981). Unfortunately pulmonary pressures were not measured in this study and it was not possible to calculate the changes within the pulmonary vasculature during the various stimuli. However it is certain that changes in pulmonary vessel diameter would be reflected in the pulmonary capillary wedge pressure, and ultimately in the cardiac output (Guyton, 1981). Despite the exact dynamics of the neurotransmitters mentioned below having yet to be described, it is possible that they were responsible for at least some of the pressure and haemodynamic changes documented in this study. These neurotransmitters will again be alluded to in the discussion on the cerebrovascular changes seen following laryngoscopy/intubation since both antidromic (Couture and Cuello, 1984; Markowitz et al, 1987) and orthodromic mechanisms (Gonzales et al, 1975; Lambert et al, 1984; Lang and Zimmer, 1974) can dilate cephalic blood vessels and increase blood flow to innervated tissues.

Neuropeptide Y is a 36 amino-acid peptide found in co-existence with noradrenaline in sympathetic nerve cell bodies and in perivascular nerve fibres associated with arteries that have an abundance of alpha-1-adrenoreceptors (Ekblad et al, 1984). Neuropeptide Y enhances adrenergically mediated contractile responses and potentiates vasoconstriction in conjunction with noradrenaline, adrenaline and histamine (Ekblad et al, 1984).

Neuropeptide Y fibres are numerous in the trachea and main bronchi where they are distributed predominantly around the blood vessels and smooth muscle bundles (Uddman et al, 1984).

Substance P is a peptide containing 11 amino acids and is present both in the central and peripheral nervous systems (Chang and Leeman, 1970). Stimulation of the respiratory mucosa, in particular the nasal mucosa, causes release of Substance P which appears to mediate vasodilatation and increased vascular permeability (Lundberg et al, 1983). The substance P fibres, derived mainly from the vagus nerve, are distributed around blood vessels and smooth muscle bundles in the trachea and main bronchi.

Vasoactive Intestinal Polypeptide (VIP) is a potent vasodilator (Said and Mutt, 1972) structurally related to the hormones secretin and glucagon. A further peptide showing striking sequence homologies with VIP is Peptide Histidine Isoleucine (PHI). PHI shares many biologic activities with VIP, although it is generally less potent (Tatemoto and Mutt, 1981). In the laryngeal region and the tracheobronchial wall, VIP/PHI fibres are numerous and close to the surface epithelium and small blood vessels (Lundberg et al, 1984). VIP innervation of the trachea and main bronchi may originate from two sources, one consisting of local intramural ganglion formations, and a second from the vagus nerve (Lundberg et al, 1978).

The peripheral vascular resistance values noted at the baseline in this study were compared with those seen in studies by Wagerle et al (1987) and Brubakk et al (1987). The baseline SVR values of the present study and the Wagerle et al (1987) study were significantly higher when compared with the baseline SVR level noted in a study by Brubakk et al (1987). One possible explanation for this is the fact that in both of the aforementioned studies hyperoxia was present. This still does explain the high SVR noted in the present study in the face of hypercarbia, since in both of the other two studies the animals were normocarbic. Because of the significant differences in baseline parameters encountered when attempting inter group comparisons within the literature, it was not possible to obtain meaningful or reliable "normal" parameter levels. It was thus difficult to assess whether the baseline SVR values seen in the present study were indicative of marked vasodilation or constriction. For this reason only changes from the arbitrary baseline have been discussed.

In the Control group there was a decrease in peripheral resistance at the 2 minute post intubation estimation, followed by a return to baseline levels that was maintained until the 15 minute reading. The increase (corresponding to the return to the baseline value) in peripheral vascular resistance in the Control group was a relatively late occurence, and could not have been responsible for the increase in blood pressure during the laryngoscopy/intubation. The hypertensive response following

laryngoscopy/intubation in both groups was almost certainly a direct result of the increased heart rate and raised cardiac output demonstrated during the stimulus. The peak heart rate was noted at approximately 2 minutes post intubation in both groups, and this corresponded to the maximal pulse pressure and cardiac output values. The peak blood pressure levels were attained some 2 minutes after the maximal heart rates, and it is likely that the cardiac output was further raised at this time. Unfortunately because of the variable nature of the response it was not possible to measure the cardiac output at the time of peak arterial pressure, but if one assumes a concommitantly raised cardiac output, then a stroke volume increase would have been essential to make up the difference, since there was no alteration in heart rate at that time. This theory is supported by the data since, despite there being no significant difference between the peak heart rate and peripheral vascular resistance of the two groups at the 2 minute estimation, the pulse pressure and cardiac output were both greater in the Control group, which may account for the difference in peak arterial pressure.

The increased pulse pressure seen during the stimulation period is indicative of a reduced vascular compliance and/or an increased stroke volume output, and thus the pressure difference noted between the times of peak heart rate and peak blood pressure response must be on the basis of a stroke volume increase occurring after the attainment of the peak heart rate. The fact that both the Control and the Lignocaine groups had similar peripheral vascular resistance levels and significantly

different pulse pressure levels at the 2 minute post intubation timepoint, suggests that the lignocaine may also cause a reduced stroke volume output.

The slight increase in mean arterial pressure seen at 15 minutes in the Control group was most likely to have been related to the increased peripheral vascular resistance at this time, compared to the immediate post-intubation level. There was no change in the arterial pressure, cardiac output or systemic vascular resistance between the 7 and 15 minute readings in the Lignocaine group, suggesting that the drug not only altered the immediate cardiac output response but also affected the delayed vascular tissue response as well.

After the 15 minute reading there was a pronounced increase in the PaCO2 and the decreased peripheral vascular resistance seen in both groups at this timepoint probably resulted from the hypercapnia. Blood pressure changed minimally over this period reflecting either a compensatory increase in cardiac output, or a direct stimulation of cardiac output by the raised PaCo2 level.

Following laryngoscopy/intubation, the lowest peripheral vascular resistance corresponded with the peak heart rate and cardiac output levels representing a maximal beta-receptor stimulation with sympathetic vasodilator influence. This peripheral vasodilation was presumably then overcome by a delayed, but more prolonged alpha receptor mediated vasoconstriction, reflected by the increase in peripheral vascular resistance in the Control group. A marked vasoconstrictor response was not seen in the

Lignocaine group. This would imply that the drug in some way attenuated the peripheral vasoconstriction, both during the insult and in the following minutes, explaining the reduced immediate hypertensive response, as well as the lower blood pressure later in the study period in the Lignocaine group.

In the lignocaine treated animals the peripheral vasculature appears to have been more dilated than in the Control group before the laryngoscopy/intubation and the additional sympathetic vasodilation following the laryngoscopy/intubation had minimal additional effect. Suplementary to this, the lignocaine may well have had a further effect which blocked the vasoconstrictor response, either at the vessel wall receptor level, or at a higher level by interfering with afferent impulse transmission. Detail of the mechanisms is not evident from the presented data, but it is likely that the reduced blood pressure response seen in the lignocaine group is strongly associated with a peripheral vasodilator action of the drug. The persistently raised cardiac output associated with a consistently lower mean arterial pressure in the Lignocaine group, as compared with the Control group, is further substantive evidence of a more pronounced and prolonged peripheral vasodilator effect in the lignocaine treated animals.

The laryngeal mechano- and chemoreceptor stimulation induced by spraying alone was noted to elicit an increase in systolic pressure (22%) which was significantly less than that seen with

laryngoscopy/spray (42%) in phase 2, suggesting that laryngoscopy is responsible for greater cardiovascular stimulation than spray alone.

The significant pressor response to spray alone indicates that either there are functioning local receptors in the respiratory mucosa which respond to contact with chemical solutions, or that the laryngeal mechanoreceptors require minimal stimulation to establish a response; the level of the hypertensive response to such mechanoreceptor stimulation being dependant on the intensity of the stimulation. From the presented data it is not possible to establish conclusively which aspect of the laryngeal stimulation produced the response during spray alone, but it is reasonable to assume that mechanical irritation accounts for the greater proportion of the blood pressure and pulse rate increase because the procedure with less mechanical stimulation excited less of a cardiovascular response. As previously discussed the reaction to the spray was not typical of the so-called laryngeal chemoreceptor "dive reflex" since the animals did not respond with apnea, bradycardia and hypertension (Grogaard et al, 1982) during the spraying of the larynx. The pure laryngeal chemoreflex was likely to have been disrupted by the mechanical stimulus of the laryngoscopy (phase 2) or the passage of the modified Forrester spray tube (phase 3), since it has been shown that beta adrenergic stimulation exerts an attenuating effect on the laryngeal chemoreflex (Grogaard et al, 1983). In addition Grogaard et al (1982) demonstrated a diminished cardiovascular response to laryngeal chemoreceptor stimulation during hyperoxia,

and they proposed that part of the response to laryngeal water administration in newborn lambs was due to arterial chemoreceptors. The animals in this study were both hyperoxic and subject to increased beta-adrenergic activity, and thus the laryngeal chemoreflex may well have been masked.

This phase 3 study demonstrates the effectiveness of topical lignocaine in modifying the cardiovascular response to laryngeal and tracheal irritation, when the drug is given at least 10 minutes before the insult.

In terms of the clinical applicability of this information, a 10 minute interval before laryngoscopy and intubation is impractical in an emergency. There are however other situations where waiting 10 minutes between spray and laryngoscopy/intubation is possible. In the intensive care milieu there are often cases where gradual decompensation of respiratory function necessitates mechanical ventilation. Oftentimes these neonates have associated problems that would be aggravated by an intense hypertensive episode. In this setting gentle laryngeal spraying 5 - 10 minutes prior to awake intubation would be possible, and in the light of the presented findings, highly advisable. A 10 minute interval between spray and intubation would also be possible in anaesthesia for elective surgery of neonates. Here the anaesthetist is often faced with newborns suffering from cardiovascular, ocular, and neurologic problems that would respond poorly to a sustained cardiovascular response.

In terms of the obstetric applications, work is planned to show that lignocaine given to the mother 10 minutes prior to delivery of an asphyxiated fetus, will protect the fetus in much the same way as demonstrated in this study.

4.4.2 Cerebrovascular

4.4.2.1 Control group

4.4.2.1.1 Laryngoscopy/intubation

Baseline brain blood flow levels in phase 3 were higher than those seen in the phase 2 study, but because of the large variances these differences were not statistically significant. The baseline flow rates were in agreement with those reported by Hansen et al (1984) for hypercarbic neonatal piglets. These authors showed brain blood flow rates with a curvilinear relationship ranging from 100 to 250 ml/min/100g in the 45 - 70 mmHg PaCO2 range. The same pattern of regional differences within a given PaCO2 range was seen in the present study as has been reported by other authors working with neonatal animals (Hansen et al, 1984; Rosenberg et al, 1982; Shapiro et al, 1980), with

the blood flow rates in the brain stem > cerebellum > cerebrum. This pattern follows the hierarchy of maturation of the newborn brain. Hypercarbia has been shown to increase brain blood flow to a greater extent in the medulla and brain stem (where blood flow levels are high in the normal developing brain) than in the cortex and deep telencephalic grey matter (Stewart, 1987; Cavazzuti and Duffy, 1981) and this was the case in this series of experiments as well.

Laryngoscopy/intubation resulted in an increase in brain blood flow in all but the two most distal brain areas, viz the medulla and the upper cervical regions. The most prominent increase was seen in the cerebral grey (61%), followed by the cerebral white (39%) and cerebellum (31%), with the flow rates in the other brain areas that showed increases, ranging from 16% to 22%. There was no change in the medulla, and an 8% reduction in the upper cervical region, the significance of these findings having been discussed above.

In all cases the greatest brain blood flow increases were seen during the laryngoscopy/intubation, before the highest blood pressure peaks were attained. Study of the tissue resistance curves revealed a pattern of initial transient vasodilatation. The only brain area that showed a significant decrease in tissue resistance during laryngoscopy/intubation was the cerebral grey matter, and in this region there was a 31% reduction in vessel resistance that corresponded with a 61% increase in cerebral grey blood flow. It is possible that many of the changes in blood

flow rate and resistance in the brain regions studied achieved statistical significance in the cerebrum and cerebellum and not in the smaller areas because of the greater tissue mass involved, and that if larger samples of the other brain areas had been harvested, the same results would have been noted. For this reason the graphs have been used to study trends and comment has been made in instances where statistical significance has not been attained, but where similar trends have been observed. Certainly, as demonstrated in the cerebral grey matter, the reduced vascular resistance combined with a rapidly rising blood pressure, allowed sudden increases in regional blood flow throughout the brain during the laryngoscopy/intubation.

Whether this initial vasodilatation is on the basis of a beta-sympathetic stimulation, a vasodilator neurotransmitter, or trigeminal nerve stimulation is not clear. Recent work by Moskowitz et al (1988) has suggested that in the steady state reflex pathways do not contribute substantially to cerebral regulation (Busija and Heistad, 1984), but that during severe perturbations such as acute hypertension, neurogenic mechanisms are more important. Because of the diffuseness of the projecting fibres, the effects of parasympathetic stimulation are less well studied. Data from work by Moskowitz et al (1988) indicates that observed cerebral vasodilatation in acute hypertension is not completely passive to increases in perfusion

pressure and that the trigeminal nerve modifies some vasomotor responses in pial precapillary vessels, promoting vasodilatation and permeability to protein.

Antidromic stimulation promotes vasodilatation and plasma extravasation and such antidromic mechanisms have been demonstrated in the trachea (Lundberg and Saria, 1982). The above mentioned neurotransmitter substances have all been implicated in the mechanism.

A second explanation of the cerebral vasodilatation may involve trigeminally mediated activation of poorly defined central connections between the trigeminal nerve and the greater superficial petrosal nerve. These fibres promote vasodilatation by cholinergic and peptidergic mechanisms (Moskowitz et al, 1988). Trigeminal nerve stimulation has been shown to decrease resistance in the external carotid artery, and this vasodilatation can be blocked by sectioning of the greater superficial petrosal nerve (Lambert et al, 1984).

Another possibility is that with the prolonged exposure to the intense stimulation seen in phase 3, there was a potent beta sympathetic outflow resulting in a temporary overwhelming of the alpha receptor mediated vasoconstriction and a degree of cerebral vasodilatation.

Whatever the mechanism this vasodilatation/reduced resistance was transient and reversed in the recovery period after intubation. Within 2 minutes the vascular resistance in some areas had increased by up to 168 % above baseline levels with

vasoconstriction in the brainstem > cerebellum > cerebrum. It is interesting to note that the greatest vasoconstriction occurred in those areas in which the least blood pressure surges were apparent viz. the brainstem and midbrain regions. These areas showed particularly efficient cerebral autoregulation with significantly reduced blood flows following intubation rather than increases during the intubation itself. This data does not agree with those of Stonestreet et al (1984) where the autoregulatory capability of the brain stem was shown to be impaired by hypercarbia. This may be partially explained by a mechanism whereby hypercarbia results in a resetting of the baseline autoregulatory threshold allowing high flow rates to develop over prolonged exposure time. Within this reset system however there may still be separate mechanisms to deal with acute surges in blood flow rate underlining the possible role played by other vasoconstrictor effectors such as the abovementioned neurotransmitters, including the prostaglandins and leukotrienes. Busija et al (1984) reported that peptidoleukotrienes can be synthesised in the brain during acute pathological conditions in neonates, and that these substances have considerable constrictor effects on the cerebral circulation. Work in this field continues but as yet no defined roles have been specified for the leukotrienes.

The return of the cerebral blood flow rates to the baseline in certain regions within 2 minutes of cessation of the stimulus cannot be explained on the basis of the systemic blood pressure, since the mean arterial pressure was still increasing at this

time. This reduction in cerebral blood flow almost certainly indicates a protective reflex vasoconstriction of the cerebral vessels, and may represent the effect of a delayed and more potent humoral response, as compared with the initial neural and neurotransmitter responses noted during the laryngoscopy and intubation. Sympathetic stimulation is known to limit cerebral vasodilatation accompanying severe abrupt increases in blood pressure within the autoregulatory range (Busija et al, 1980). With increases above the limits of autoregulation, sympathetic stimulation blunts the expected increases in cerebral blood flow (Bill and Linder, 1976). This is the presumed mechanism for the sudden vasoconstriction noted following the initial vasodilatation. The presence of alpha-1-adrenoreceptors in the cerebral circulation has been dealt with previously in this thesis (Wagerle and Delivoria-Papadopoulos, 1987).

The significant cerebral vasodilatation associated with raised cerebral blood flow noted at the 15 and 20 minute timepoints in the cerebral grey matter is most probably the result of the worsening hypercarbia seen in these animals towards the end of the experiment. The cerebrum is known to be less sensitive to hypercarbia than other brain areas (Hansen et al, 1984; Brubakk et al, 1987) and thus only at higher PaCO2 levels does the autoregulation become impaired to the degree that blood flow rates increase.

When these results are compared with those seen in phase 2, it appears that the stimulation of the laryngoscopy/intubation caused significant increases in brain blood flow in the initial few seconds of stimulation. A reduced vascular resistance combined with an increasing blood pressure induced by laryngoscopy/intubation tended to increase the brain blood flow. This may be the result of a specific mechanism induced by the stimulation of the trachea and subglottic structures. However this picture may simply reflect a "breakthrough" indicating the initial inability of the vasoconstrictor autoregulation to cope with the rapidly rising cerebral perfusion pressure. In phase 3 the laryngoscopy/intubation microsphere shoot was performed at least 30-40 seconds before the estimation in phase 2. The cerebral autoregulatory response was possibly better established in the phase 3 animals at the time of brain blood flow estimation explaining the observed difference in blood flow rate between the two groups following the stimulus. However, as discussed earlier it is difficult to extrapolate phase 2 results into the phase 3 situation because of design differences.

The cerebrovascular effects of the various neurotransmitter substances released during the type of stimulation of the respiratory mucosa investigated in this study, have not been reported in neonates. From the previous discussion of the varied vascular effects of these substances it seems possible that laryngoscopy (upper airway and pharyngeal stimulation) may excite the release of a vasoconstrictor substance, but that more distal

irritation of the respiratory tree (subglottic and tracheo-bronchial stimulation) as seen with intubation, results in the release of vasodilator compounds that may counter or reverse the vasoconstriction. There is still much to be learned in terms of the detailed release patterns and effects of these neurotransmitter substances following the acute stimulation of the larynx, pharynx and trachea.

In almost all of the brain areas the blood flow had stabilized and was again insignificantly different from the baseline values within 7 minutes. The only region showing a persistently reduced blood flow was the upper cervical area.
This data supports the hypothesis that the stimulation of laryngoscopy and intubation has profound and in some cases prolonged effects on the cerebral vasculature. Under certain circumstances these blood flow changes may well lead to pressure surges, extravasation of protein into the tissues (with all of its attendant problems), and even disruption of the vascular lining and haemorrhage.

The presence of bleeding in the midbrain and brain stem in phase 1 is difficult to explain in the light of the findings of phase 2 and 3. The most active and efficient cerebral autoregulation was shown to occur in the area of most apparent bleeding. A possible explanation for this may lie in the fact that the blood flow in the midbrain and basal areas of the brain was already significantly elevated in these animals due to pre-existing

hypercarbia. Hansen et al (1984) and Brubakk et al (1987) showed that cerebral blood flow in the brain stem was increased to a greater extent than in other areas of the brain during hypercarbia. There is consensus amongst a number of workers in this field as to baseline brain region blood flow rates during normocarbia, with flow rates ranging from 41 to 104 ml/min/100g for the various regions (Hansen et al, 1984; Brubakk et al, 1987; Wagerle et al, 1987). When one compares the cerebral regional blood flow rates noted in this study with those reported in the literature, an immediate differential increase is noted. There was a significantly higher baseline blood flow rate in the brainstem, midbrain and cerebellum ($p < 0.05$,) regions in the present study (hypercapnia) when compared with the baseline normocapnic levels of Brubakk et al (1987). The cerebrum blood flow rates were not significantly different despite the blood gas differences. The blood flow rates in the same areas during hypercarbia in the Brubakk et al (1987) study were not different from the baseline flow rates seen in this study. Thus at the baseline measurements in this study, blood flow rates were already significantly higher in the brainstem (200%) and midbrain (102%) than in normocarbic animals, while the cerebrum blood flow rates were minimally changed. The subsequent increases in blood flow rate in the basal areas noted during laryngoscopy/ intubation, despite being smaller than those in the cerebrum, may still have been sufficient to induce vascular disruption in an already strained system. This finding is important since it may explain the high proportion of brain bleeds in the basal area.

The major proportion of the blood flow increases were thus seen to be due to the raised PaCo2 level. The fact still remains that there were more bleeds in the intubated group and this must indicate that the additional stimulation of laryngoscopy/ intubation contributes to the pathology. It may well be that during the phase 1 study the vascular integrity in the basal brain areas was precariously balanced during hypercarbia, and that despite the tremendously increased flow rate the blood vessels remained intact in the Control animals. The same vessels were however unable to withstand the sudden increase over their threshold level during laryngoscopy/intubation, explaining the higher proportion of bleeds in the stimulated group. The threshold level of blood flow for vascular disruption may be only minimally higher than the baseline level during this level of hypercarbia, and thus only the slightest perturbation may potentially lead to bleeding.

An important clinical application of this finding is that laryngoscopy and intubation in hypercarbic neonates may well be potentially more damaging to the basal brain than the same procedure in normocarbic newborns because of the combination of the effects of the disproportionately increased blood flow rate (from the raised PaCO2), and the additional stress of the laryngoscopy/intubation. Obviously the brain blood flow changes in normocarbic animals under the same circumstances need to be determined before this hypothesis can be stated with any

certainty. Should this be the case then the routine manual hyperventilation of hypercarbic neonates prior to intubation would assume an even more important role than the prevention of hypoxia, viz. the reduction of the basal brain blood flow rate, and the possible prevention or reduction of bleeding in this area following laryngoscopy and intubation.

4.4.2.2 The effect of Lignocaine

Animals treated with lignocaine showed two different patterns of blood flow and vascular resistance response to laryngoscopy/ intubation. In the cerebral grey matter, cerebral white matter and cerebellum (low-flow areas) there was, during laryngoscopy/intubation in the Lignocaine group, an initial rapid increase in blood flow with an associated decrease in vascular resistance that closely mirrored the changes seen in the Control group. This was followed by a significantly elevated vascular resistance that was similar in both groups, but a reduction in blood flow that was much more pronounced in the Lignocaine group. In the other brain regions the different vascular response was immediately apparent, since in almost all regions there was a higher vascular resistance and lower blood flow rate in the

Lignocaine group than in the Control group during the laryngoscopy/intubation. This was followed by a gradual return to baseline values. In most brain areas the blood flow rates in the Lignocaine group were reduced below the Control group levels for up to 15 minutes. These different patterns of response are well illustrated in the flow and resistance graphs. In addition to the difference in behaviour between the different tissue regions over the laryngoscopy/intubation period, there was also a tendency for a lower flow rate in the Lignocaine group when assessed over the whole study period. At the 2 minute measurement in all tissue regions except for medulla, caudate nucleus and pons, the blood flow in the Lignocaine group was lower than in the Control group.

There is a paucity of published data on the cerebral circulatory effects of lignocaine, and mechanisms to explain these findings are not immediately evident.

Lignocaine may act at the mucosal level blocking the transmission of cholinergic and peptidergic impulses from the respiratory mucosa, thus blunting vasodilatatory impulses during the stimulation. The effects of this would be to attenuate the intra laryngoscopy/intubation blood flow surge by limiting vessel diameter during the insult, and to potentiate the post laryngoscopy/intubation vasoconstriction. In the high-flow brain regions there was a significant increase in vascular resistance in the Lignocaine group during the irritation, which was not seen in the Control group. This difference in response disappeared within 2 minutes supporting a local neural mechanism since the

rapidity of the changes would tend to exclude a central humoral mechanism. The lack of this autoregulatory response in the low-flow group may be the result of failure to surpass a threshold blood flow rate during the stimulus.

Lignocaine has been found in arterial blood within 1 minute of laryngotracheal administration (Viegas and Stoelting, 1975) and maximum levels after such spraying are reported at times ranging from 5 minutes (Adriani and Campbell,1956; Pelton et al, 1970), to 9 - 15 minutes (Viegas and Stoelting, 1975), to 15 -20 minutes (Chu et al, 1975) and to 20 -30 minutes (Rosenberg et al, 1980). Sakabe et al (1974) showed that there was a negligible blood-brain barrier for lignocaine in dogs, with peak CSF levels appearing within 6 minutes of peak arterial levels. It is thus likely that lignocaine exerted some central cerebrovascular effect during laryngoscopy/intubation. It has been suggested that lignocaine causes a depression of the autoregulatory mechanism, thereby allowing excessive drops in cerebral perfusion pressure during times of reduced mean arterial pressure (Sakabe et al, 1974). This hypothesis is however not supported by the data from several of the brain regions in this study, since there was a significantly greater degree of vasoconstriction in the medulla, midbrain, thalamus and cerebellum regions of the Lignocaine group during laryngoscopy/intubation, and at the 7 minute timepoint, indicating a more reactive cerebral vasculature than in the Control group.

The impression gained from the results of this study is that lignocaine tends to sensitise the cerebral vasculature to vasoconstrictive influences. This may be on the basis of a local tracheal mechanism as previously discussed, or may be the result of a regional effect of the drug, with specific areas of the brain showing greater sensitivity than others. The fact that the mean arterial pressure rose to the same degree in both groups during the laryngoscopy/intubation, but was associated in the Lignocaine group with significantly higher vascular resistances and lower flow rates in the areas mentioned, is indicative of more reactive vessels in these brain regions. This implies that not only were there inter group differences, but also that within the Lignocaine group there was varied sensitivity to the drug.
The drug did not appear to have direct vasoconstrictor effects, since during the baseline and 20 minute measurements, there were no significant differences in vascular resistance between the two groups. The lignocaine did not increase the intensity of the vasoconstriction, but rather accelerated the response time and prolonged the duration of the effect, supporting the theory that some undefined vasodilatory mechanism was inhibited. The effect of hyperoxia on the response of the cerebral vessels to lignocaine should not be ignored since there may well have been an interaction betwen the drug and the vasoconstrictive propensity of the elevated blood oxygen tension in the cerebral vessels.

Although lignocaine is generally thought of as a vasodilating drug, and at high concentrations is known to relax all vascular smooth muscle, it is not impossible that under certain circumstances it may have different effects in different vascular beds. As mentioned in the introduction, isolated rat portal vein (Klein et al, 1968), rat mesocaecum arterioles (Altura, 1967), canine pulmonary vein segments (Hyman, 1970), pregnant human uterine artery (Cibils, 1976) and rat cerebral arterioles (Lescanic et al ,1981) have all been shown to constrict in response to lignocaine. Lescanic et al (1981) demonstrated decreased brain blood flow in rats exposed to blood levels of 6.43 micrograms/ml of lignocaine. The mechanism by which lignocaine potentiates vasoconstriction and reduces brain blood flow is unknown.

There appeared to be a disparity in the response seen following laryngoscopy/intubation in the lignocaine treated animals in phase 2 compared to that noted in phase 3. Because of the different baseline milieu, the different timepoints at which the flow rate was estimated, and the single data point in phase 2 (which is impossible to accurately relate to an equivalent timepoint in the phase 3 study) it is not possible to comment on this apparent difference. The commentary on the effects of lignocaine on the response of the brain blood flow to the stimulus of laryngoscopy/intubation has thus been confined to comment on the phase 3 data.

1 Conclusions

1. The stimulus of laryngoscopy/intubation is causally related to the development of histologically evident, non-lethal cerebral haemorrhage and protein extravasation in hyperoxic, hypercapnic newborn piglets.

These bleeds are mainly situated in the basal areas of the brain, a region not previously shown to be at risk during such intervention. The potential for long term morbidity resulting from bleeding in this area of the brain is great. The number of patients developing neurodevelopmental and behavioural abnormalities following situations that required laryngoscopy/ intubation is significant, and any factor that might contribute to such permanent disability should be thoroughly investigated - especially if alteration of the current management protocols to minimise some or all of the injurious effects, is possible.

2. Laryngoscopy/intubation is associated with significant increases in heart rate, blood pressure, pulse pressure, and cardiac output. These increases are in some instances prolonged for up to 12 minutes, indicating that the insult is neither transient nor localized, and that more attention should be focussed on the possible delitarious cardiovascular effects of this seemingly innocuous procedure.

There are changes in vascular resistance induced by the stimulus that, in many instances, are prolonged for up to 7 minutes. Laryngoscopy/intubation appears to cause a transient reduction in peripheral vascular resistance that lags behind the event itself, and most probably represents a compensation for the hypertension. Possible causes for the observed changes have been suggested, including sympathetic vasodilatation, and the contribution of some newly isolated neurotransmitter substances.

3. Cerebral blood flow is significantly increased above baseline levels by hypercarbia. The major increases in blood flow are noted in the brainstem, and the findings of this study are in agreement with those of other workers in this field.

Laryngoscopy/intubation is associated with increases in blood flow in almost all areas of the brain, with the most significant increases occurring in those regions least affected by hypercarbia, viz cerebrum and cerebellum. In most cases the brain blood flows return to the baseline within 2 minutes. Despite the smaller percentage changes, in hypercarbic piglets the absolute blood flow rates following laryngoscopy/intubation are significantly greater in the diencephalon and brainstem than in the cerebrum, and the preponderance of pathology in the aforementioned areas may be a result of this.

4. The cerebral autoregulatory mechanisms in hypercarbic piglets are not entirely effective in preventing "breakthrough" surges of brain perfusion pressure, and this study has demonstrated that laryngoscopy/intubation is a stimulus that is capable of causing such uncontrollable increases in brain blood flow. Although little data exists on the cerebral autoregulatory response to sympathetic stimulation in term neonates, there is evidence to suggest that hypercarbia may cause impairment.

5. The results of this study suggest that there may be several interelated mechanisms responsible for cerebral autoregulation, with different threshold levels for absolute and relative blood flow changes. In addition these mechanisms may undergo constant resetting depending on the ambient environment and the rapidity of change of this environment. This may result in different responses of the cerebral vasculature to acute and chronic increases in blood flow rate.

6. Laryngoscopy and endotracheal intubation may be a more damaging stimulus in hypercarbic neonates than in normocarbic newborns. This is because of the disproportionately increased brain blood flow rate in the basal region of the brain caused by the raised carbon dioxide tension. The additional increase in blood flow following the stimulus leads to pressures that may exceed the containing capabilities of the vascular walls in these vessels, with resultant protein extravasation and haemorrhage.

Although the brain blood flow changes after laryngoscopy/ intubation in normocarbic neonates have not as yet been determined, by extrapolation of the results of other researchers who have studied the effects of carbon dioxide on neonatal cerebral blood flow, this hypothesis is reasonable.
It has been recommended that all neonates should have a period of hyperventilation with 100% oxygen prior to laryngoscopy and intubation (Hinkle, 1986). From the results of this work such a procedure would correct any underlying hypoxia and possibly more importantly, reduce any hypercarbia that may be present.

7. Many of the changes induced by laryngoscopy/intubation have been demonstrated to be significantly altered by the topical spray of the local anaesthetic agent lignocaine in the laryngopharynx prior to the procedure. Cardiovascular responses are blunted in treated animals, with less of a mean arterial blood pressure response, and a shorter duration of effect.

8. The surge of hypertension noted in many brain regions during laryngoscopy/intubation is prevented by the use of lignocaine spray.

9. The use of topical lignocaine in neonatal piglets does not cause dangerous myocardial depression and is not accompanied by any immediately obvious adverse side-effects.

10. It is suggested that the elective use of topical lignocaine prior to laryngoscopy/intubation in selected cases may reduce the ill-effects of the procedure. Whether the routine use of this drug will prevent cerebral haemorrhage is not known, but by reducing the hydrostatic pressure within the cerebral vasculature the chances of haemorrhage may be diminished. Lower cerebral perfusion pressures in a vasculature made vulnerable by previous insults, may reduce the degree of protein extravasation into the interstitial tissue, thus decreasing the incidence of cerebral oedema. This may well be the most important benefit of minimising the hypertensive surge seen with laryngoscopy/intubation, especially in already compromised neonates.

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Abildskov, J.A. Adrenergic effects on the Q-T interval of the electrocardiogram. Am. Heart J. 92:210-216, 1976

Abou-Madi, M., Keszler, H. and Yacoub, O. A method for prevention of cardiovascular reactions to laryngoscopy and intubation. Canad. Anaesth. Soc. J. 22:316-329, 1975

Abou-Madi, M., Keszler, H. and Yacoub, O. Cardiovascular reactions to laryngoscopy and tracheal intubation following small and large intravenous doses of lidocaine. Can. Anaesth. Soc. J. 24:12-19, 1977

Adriani, J. and Campbell, D. Fatalities following topical application of local anesthetics to mucous membranes. JAMA 162:1527-1530, 1956

Alexander, S.C. and Lassen, N.A. Cerebral circulatory response to acute brain disease. Anesthesiology 32:60-68, 1970

Altura, B.M. Evaluation of neurohumoral substances in local regulation of blood flow. Am. J. Physiol. 212:1447-1454, 1967

Andren, B. Arterial acid-base measurements in 1-3 days old piglets. Acta. Vet. Scand. 23:581-591, 1982

Atkinson, R.S., Rushman, G.B. and Lee, J.A. (1982) Inhalational Anaesthesia. In: A Synopsis of Anaesthesia, Bristol, Wright.PSG, p.p. 168-217.

Ayre, T.P. Theme and variation (On a T-piece). Anaesthesia 22: 359, 1967

2 B

Barash, P.G., Kopriva, M.D. Giles, R., Tarabadkar, S., Berger, H and Zaret, B. Global ventricular function and intubation: Radionuclear Profiles. Anesthesiology 53:S109, 1980

Baumbach, G.L. and Heistad, D.D. Effects of sympathetic stimulation and changes in arterial pressure on segmental resistance of cerebral vessels in rabbits and cats. Circ. Res. 52(5):527-533, 1983

Bedford, R.F., Winn, H.R. and Tyson, G. Lidocaine prevents increased ICP after endotracheal intubation. Intracranial Pressure IV. Edited by Shulman, K., Mamarou, A., Millar, J.D. Berlin, Springer, 1980, pp 595-598

Bedford, R.F. and Marshall, W.K. Haemodynamic response to endotracheal intubation: Four anesthetics. Anesthesiology 55:A270, 1981

Bernstein, J.S., Nelson, M.A., Ebert, T.J., Woods, M.P. and Roerig, D.L. Beat-by-beat cardiovascular responses to rapid sequence induction in humans: Effects of labetalol. Anesthesiology 67:A32, 1987

Berry, C.A., Sanner, J.H. and Keasling, H.H. A comparison of the anticonvulsant activity of mepivicaine and lidocaine. J. Pharmacol.Exp. Ther. 133:357-363, 1961

Berry, F.A., Gregory, G.A. Do premature infants require anaesthesia for surgery? Anesthesiology 67:291-3, 1987

Betz, A.L. and Goldstein, G.W. Changes in biochemical properties of isolated brain capillaries. Pediatr. Res. 13:355, 1979

Bevan, J.A., Duckles, S.P. and Lee, T.J-F. Histamine potentiation of nerve- and drug-induced responses of rabbit cerebral artery. Circ. Res. 36:647-653, 1975

Biehl, D.R., Cote, J., Wade, J.G. and Gregory, G.A. Uptake of halothane in the fetal lamb in utero. Anesthesiology 53:S310, 1980

Bill, A. and Linder, J. Sympathetic control of cerebral blood flow in acute arterial hypertension. Acta. Physiol. Scand. 96:114-121, 1976

Blayaert, A. Letter. Anesthesiology 49:390, 1978

Boggs, D.F. and Bartlett, D. Jnr. Chemical specificity of a laryngeal apneic reflex in puppies. J. Appl. Physiol. 53:455-462, 1982

Bourne, J.G. Thiopentone-nitrous oxide-oxygen anaesthesia with curare for head and neck surgery. Br. Med. J. 2:654-655, 1947

Boyd, J.D. The inferior aortico-pulmonary glomus. Brit. Med. Bull. 17:127-131, 1961

Brady, J.P., Cotton, E.C. and Tooley, W.H. Chemoreflexes in the Newborn Infant: Effects of 100% oxygen on heart rate and ventilation J. Physiol. (Lond.) 172:332-341, 1964

Bromage, P.R. and Robson, J.G. Concentrations of Lignocaine in the blood after intravenous, intramuscular, epidural and endotracheal administration. Anaesthesia 16:461-478, 1961

Brubakk, A., Oh, W. and Stonestreet, B.S. Prolonged hypercarbia in the awake newborn piglet: Effect on brain blood flow and cardiac output. Pediatr. Res. 21:29-33, 1987

Brubakk, A-M., Oh, W. and Stonestreet, B.S. Adaptation of brain blood flow to prolonged hypercarbia in the newborn piglet. Pediatr. Res. 71:361A, 1983

Brubakk, A-M., Bratlid, D., Oh, W. and Stonestreet, B.S. The effect of hypercarbia and hypertension on brain blood flow in the newborn piglet. Pediatr. Res. 71:361A, 1983

Brubakk, A., Bratlid, D., Oh, W., Yao, A., and Stonestreet, B. Atropine prevents increases in brain blood flow during hypertension in newborn piglets. Pediatr. Res. 18: 1121-1126, 1985.

Bruhn, F.W., Mokrohisky, S.T., and McIntosh K.: Apnea associated with respiratory syncitial virus infection in young infants. J. Pediatr., 90:382-386, 1977.

Buckberg,G.D., Luck,J.C., Payne,B.D., Hoffman,J.I.E., Archie,J.P., Fixler,D.E. Some sources of error in measuring regional blood flow with radioactive microspheres.
J. Appl. Physiol. 31:598-604, 1971.

Buckley, N. Maturation of the circulatory system in three mammalian models of human development. Comp. Biochem. Physiol. 83A:1-7, 1986

Buckley, N., Brazeau, P. and Gootman, P. M. Maturation of circulatory responses to adrenergic stimuli. Fed. Proc. 42:1643-1647, 1986

Burney, R.G. and Winn, R. Increased cerebrospinal fluid pressure during laryngoscopy and intubation for induction of anesthesia. Anesth. Analg. 54:687-690, 1975

Burnstock, G. Purinergic nerves. Pharmacol. Rev. 24:509-580, 1972

Burstein, C.L., LoPinto, F.J. and Newman, W. Electrocardiographic studies during endotracheal intubation. !. Effects during usual routine technics. Anesthesiology 11:224-237, 1950

Burstein, C.L., Woloshin, G. and Newman, W. Electrocardiographic studies during endotracheal intubation. 11. Effects during general anaesthesia and intravenous procaine. Anesthesiology 11:229-241, 1950

Busija, D.W., Heistad, D.D. and Marcus, M.L. Effects of sympathetic nerves on cerebral vessels during acute, moderate increases in arterial pressure in dogs and cats. Circ. Res. 46:696-702, 1980

Busija, D. and Heistad, D.D. Factors involved in the physiological regulation of the cerebral circulation. Rev. Physiol. Biochem. Pharmacol. 101:162-211, 1984

Busija, D., Leffler, C.W. and Beasley, D.G. Peptidoleukotrienes (LTC4, D4, E4) constrict cerebral arteries of piglets. Ped. Res. 34:344A, 1986

Busija, D.W., Leffler, C.W. and Wagerle L.C. Responses of newborn pig pial arteries to sympathetic nervous stimulation and exogenous norepinephrine. Pediatr. Res. 19: 1210-1214, 1985

Busija, D.W. and Leffler, C.W. Postjunctional alpha-2 adrenoceptors in pial arteries of newborn pigs. J. Dev. Pharmacol. Ther. 10:36-46, 1987

Busija, D.W., Orr, J.A., Rankin, J.H.G., Liang, H.K. and Wagerle, L.C. Cerebral blood flow during normocapnic hyperoxia in the unanesthetised pony. J. Appl. Physiol. Respirat. Environ. Excersize Physiol. 48 (1):10-15, 1980

3 C

Cavazzuti, M. and Duffy, T.E. Regulation of local cerebral blood flow in normal and hypoxic newborn dogs. Ann.Neurol. 11:247-257, 1981

Chang, M. M. and Leeman, S.E. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J. Biol. Chem. 245:4784-4790, 1970

Charlton, A.J. and Greenhough, S.G. Blood pressure response of neonates to tracheal intubation. Anaesthesia 43:744-746, 1988

Chraemmer-Jorgensen, B., Hoilund-Carlsen, P.F., Marving. J. and Christensen, V. Lack of effect of intravenous lidocaine on hemodynamic responses to rapid sequence induction of general anesthesia: a double-blind controlled clinical trail. Anesth. Analg. 65;1037-1041, 1986

Christensen, M.S., Hoedt-Rasmussen, K. and Lassen, N.A. Cerebral vasodilatation by halothane anaesthesia in man and its potentiation by hypotension and hypercapnia. Br. J. Anaesth. 39:927-934, 1967

Chu, S.S., Rah, K.H., Brannan, M.D. and Cohen, J.L. Plasma concentrations of lidocaine after endotracheal spray. Anesth. Analg. (Cleve) 54:438-442, 1970

Chung, K.S., Sinatra, R.S., Harrison, D.M. and Chung, J.H. Does intermediate dose labetalol block the heart rate and blood pressure responses to laryngoscopy and intubation? A placebo controlled study. Anesthesiology 69:A43, 1988

Cibils, L.A. Responses of human uterine arteries to local anesthetics. Am. J. Obstet. Gynecol. 126:202-210, 1976

Collinsworth, K.A., Kalman, S.M. and Harrison, D.C. The clinical pharmacology of lidocaine as an anti-arrhythmic drug. Circulation 50:1217-1230, 1974

Corbett, J.L., Kerr, J.H., Prys-Roberts, C. Cardiovascular responses to aspiration of secretions from the respiratory tract in man. J. Physiol.(Lond) 20:51-52, 1969

Couture, R. and Cuello, C. Studies on the trigeminal antidromic vasodilatation and plasma extravasation in the rat. J. Physiol. Lond. 346:273-285, 1984

4 D

Davies, M.J. Cronin, K.D. and Cowie, R.W. The prevention of hypertension at intubation. A controlled study of intravenous hydrallazine on patients undergoing intracranial surgery. Anaesthesia 36:147-151, 1981

Davison, M.H.A. Letter. Br.J.Anaesth. 23:238, 1951

Dawes, G.S., Johnston, B.M. and Walker, D.W. Relationship of arterial pressure and heart rate in foetal, newborn and adult sheep. J. Physiol. London 309:405-418, 1980

Dawson, B., Michenfelder, J.D. and Theye, R.A. Effects of ketamine on canine cerebral blood flow and metabolism. Modification by prior administration of thiopental Anesth. Analg. (Cleve.) 50:443-447, 1971

Denlinger, K.J., Ellison, N. and Ominsky, A. Effects of intratracheal lidocaine on circulatory responses to tracheal intubation. Anesthesiology 41:409-412, 1974

Derbyshire, D.R., Chmielewski, A., Fell, D., Vater, M., Achola, K. and Smith G. Plasma catecholamine responses to tracheal intubation. Br. J. Anaesth. 55:855-859, 1983.

Derbyshire, D.R., Smith, G. and Achola, K.J. Effect of topical lignocaine on the sympathoadrenal responses to tracheal intubation. Br. J. Anaesth. 59:300-304, 1987

Deutsch, S., Linde, H.W., Dripps, R.D. and Price, H.L. Circulatory and respiratory actions of halothane in normal man. Anesthesiology 23:631-638, 1962

DeVault, M., Greifenstein, F.E. and Harris, L.C. Circulatory responses to endotracheal intubation in light general anesthesia - the effect of atropine and phentolamine. Anesthesiology 21:360-362, 1960

Dingle, H.R. Antihypertensive drugs and anaesthesia. Anaesthesia 21:151-172, 1966

Dohi, S., Nishikawa,T., Ujike, Y., Mayumi, T. Circulatory responses to airway stimulation and cervical epidural blockade. Anesthesiology 57:359-363, 1982

Dohi, S. and Gold, M.I. Pulmonary mechanics during general anaesthesia. The influence of mechanical irritation on the airway. Br. J. Anaesth. 51:205-124, 1979

Dobbing, J. and Sands, J. Comparative aspects of the brain growth spurt. Early Hum. Dev. 3:79-83, 1979

Donat, J.F., Okazaki, H., and Kleinberg, F. Intraventricular hemorrhages in full-term and premature infants. Mayo Clin. Proc. 53:437-441, 1978

Donegan, M.F. and Bedford, R.F. Intravenously administered lidocaine prevents intracranial hypertension during endotracheal suctioning. Anesthesiology 52:516-518, 1980

Downes, J.J., Kemp, R.A. and Lambertsen, C.J. The magnitude and duration of respiratory depression due to fentanyl and meperidine in man. J. Pharmacol. exp. Ther. 158:416-420, 1967

Durand, M., Sangha, B., Cabal, L.A., Hoppenbrouwers, T. and Hodgman, J.E. Cardiopulmonary and intracranial pressure changes related to endotracheal suctioning in preterm infants. Crit. Care Med. 17:506-10, 1989

5 E

Editorial. Pain , anaesthesia and babies. Lancet 2:543-4, 1987

Edvinsson, L. Neurogenic mechanisms in the cerebrovasular bed. Autonomic nerves, amine receptors, and their effects on cerebral blood flow. Acta. Physiol. Scand. Suppl. 427:1-35, 1975

Edvinsson, L. and MacKenzie, E.T. Amine mechanisms in the cerebral circulation. Pharmacol. Rev. 28:275-348, 1973

Edvinsson, L. and Sercombe, S. Influence of pH and pCO2 on alpha receptor contraction in brain vessels. Acta. Physiol. Scand. 97:325-331, 1976

Ekblad, E., Edvinsson, L., and Uddman, R. Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibres. Regul. Pept. 8:225-235, 1984

Eschenbacker, W.L., Boushey,H.A., and Sheppard,D.: Alteration in osmolarity of inhaled aerosols causes brochoconstriction and cough, but absence of a permeant anion causes cough alone. Am. Rev. Respir. Dis. 129:211-215, 1984.

Eyzaguirre, C. and Lewin, J. Chemoreceptor activity of the carotid body of the cat. J. Physiol. (Lond.) 159:222-237, 1961

6 F

Fink, H.R. and Haschke, R.H. Anesthetic effects on cerebral metabolism. Anesthesiology 39:199-215, 1973

Fisher, D.M., Frewen, T. and Swedlow, B.D. Increase in intracranial pressure during suctioning - stimulation vs rise in PaCO2. Anesthesiology 57:426-431, 1982

Flacke, W. and Alper, M.H. Actions of halothane and norepinephrine in the isolated mammalian heart. Anesthesiology 23:793-801, 1962

Forbes, A.M. and Dally, F.G. Acute hypertension during induction of anaesthesia and endotracheal intubation in normotensive man. Br. J. Anaesth 42:618-624, 1970

Forrester, A.C. Redesigned Forrester spray. Br. J. Anaesth. 46:413, 1974.

Friedman, W.F. The intrinsic physiologic properties of the developing heart. Prog. Cardiovasc. Dis. 15:87-111, 1972

Friesen, R.H., Henry, D.B. Cardiovascular changes in preterm neonates receiving isoflurane, halothane, fentanyl and ketamine. Anesthesiology 64:238-42, 1986

Friesen, R.H., Honda, A.T. and Thieme R.E. Changes in anterior fontanel pressure in preterm neonates during tracheal intubation. Anesth. Analg. 66:874-878, 1987

7 G

Gallagher,J.T., Kent,P.W., Passatore,M.: The composition of tracheal mucous and the nervous control of its secretion in the cat. Proc. R. Soc. London.(Biol.),192:49-76, 1975.

Gibbs, J.M. The effects of endotracheal intubation on cardiac rate and rhythm. NZ. Med. J. 66:465-469, 1967

Ginn, R., and Vane,J.R. Disappearance of catecholamines from the circulation. Nature(Lond.), 219:740-742, 1968

Glawischnig, E. and Schlerka, G. Verlaufsuntersuchungen uber die Blutgase und den Saure-basen-Haushalt mit Bestimmung von Haematokrit und Haemoglobin bei Verkeln. D.T.W. 87:441-443, 1980

Goddard, J., Lewis, R.M., Armstrong, D.L. and Zeller, R.S. Moderate, rapidly induced hypertension as a cause of intraventricular hemorrhage in the newborn beagle model. J. Pediatr. 96:1057-1060, 1980

Gonzales, G., Onofrio, B.M. and Kerr, F.W.L. Vasodilator system for the face. J. Neurosurg. 42:696-703, 1975

Goodman, L.S. and Gilman, A. Local Anesthetics. In: The Pharmacological Basis of Therapeutics. Cocaine; Procaine and other synthetic local anesthetics. New York: Macmillan, 1975, chapter 20, p. 379-403.

Gootman, N., Gootman, P.M., Buckley, N.M., Cohen, M.I., Levine, M. and Spielberg, R. Central vasomotor regulation in the newborn piglet Sus Scrofa. Am. J. Physiol. 222:994-999, 1972

Goplerud, J.G., Wagerle, C.W. and Delivoria-Papadopoulos M. Role of sympathetics in regional cerebral blood flow response to apnea in newborn piglets (Abstract). Federation Proc. 46:643, 1983

Gotoh, F., Tazaki, Y and Meyer, J.S. Transport of gases through brain and their extravascular vasomotor action. Exp. Nerol. 4:48-58, 1961

Greenbaum, R., Cooper, R., Hulme, A. and Mackintosh, I.P. The effect of induction of anaesthesia on intracranial pressure. In Recent advances in Anesthesiology and Resuscitation (ed. A. Arias), p. 794 Excerpta Medica, Amsterdam.

Grogaard, J., Lindstrom, D.P., Marchal, F., Stahlman, M.T. and Sundell, H. The cardiovascular response to laryngeal water administration in young lambs. J. Dev. Physiol. 4:353-370, 1982

Grogaard, J. and Sundell, H. Effect of beta-adrenergic agonists on apnea reflexes in newborn lambs. Pediatr. Res. 17:969-972, 1983

Guyton, A.C. Part V: The Circulation. In Guyton, A.C., Textbook of Medical Physiology, Philadelphia, W.B.Saunders Co., 1981

8 H

Haggendal, E. Blood flow autoregulation of the cerebral grey matter with comments on its mechanism. Acta. Neurol. Scand. 41(Suppl.14):104+, 1965

Haggendal, E. Effects of some vasoactive drugs on the vessels of cerebral grey matter in the dog. Acta. Physiol. Scand. 66(Suppl. 258):55-79, 1966

Haggendal, E., Johansson, B. On the pathophysiology of the increased cerebrovascular permeability in acute arterial hypertension in cats. Acta. Neurol. Scand. 48:265-270, 1972

Hales, J.R.S. Radioactive microsphere techniques for studies of the circulation. Clin. Exp. Pharmacol. Physiol. 1(Suppl): 31-46, 1974.

Hambleton, G and Wigglesworth, J.S. Origen of intraventricular haemorrhage in the preterm infant. Arch. Dis. Child. 51:651-659, 1976

Hamil, J.F., Bedford, R.F., Weaver, D.C. and Colohan, A.R. Lidocaine before endotracheal intubation: Intravenous or laryngotracheal? Anesthesiology 55:578-581, 1981

Hansen, N.B., Brubakk, A., Bratlid, D., Oh, W., and Stonestreet,B. The effects of variations in PaCO2 on brain blood flow and cardiac output in the newborn piglet. Pediatr. Res. 18: 1132-1136, 1984.

Hansen, T.D., Warner, D.S. and Todd, M.M. Nitrous oxide is a more potent cerebral vasodilator than either halothane or isoflurane. Anesthesiology 69:A537, 1988

Harding,R., Johnson, P., and McClelland, M.E.: Liquid-sensitive laryngeal receptors in the developing sheep, cat and monkey. J. Physiol. (Lond.), 277:409-422, 1978.

Harper, A.M. and Bell, R.A. The effect of metabolic acidosis and alkalosis on the blood flow through the cerebral cortex. J. Neurol. Neurosurg. Psychiatry 26:341-344, 1963

Harper, A.M. and Glass, H.I. Effect of alterations in the arterial carbon dioxide tension on the blood flow through the cerebral cortex at normal and low arterial blood pressures. J. Neurol. Neurosurg. Psychiatry 28:449-452, 1965

Harrison, D.C., Sprouse, H.J. and Morrow, A.G. The antiarrythmic properties of lidocaine and procaine amide: Clinical and physiologic studies of their cardiovascular effects in man. Circulation 28:486-491, 1963

Hauswirth, O. and Schaer, H. Effects of halothane on the sino-atrial node. J. Pharmacol. Exp. Ther., 158:36-39, 1967

Heistad, D.D. and Marcus, M.L. Evidence that neural mechanisms do not have important effects on cerebral blood flow. Circ. Res. 42:295-302, 1978

Heistad, D.D. and Marcus, M.L. Effect of sympathetic stimulation on permeability of the blood-brain barrier to albumin during acute hypertension in cats. Circ. Res. 45:331-338, 1979

Heistad, D.D., Marcus, M.L., Ehrhardt, J.C. and Abboud, F.M. Effect of stimulation of carotid chemoreceptors on total and regional cerebral blood flow. Circ. Res. 38:20-25, 1976

Heistad, D.D. and Kontos, H.A. Cerebral Circulation. In: Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow. Bethesda, MD: Am. Physiol. Soc., 1984, sect. 2, vol. III, chapt. 5, p. 137-182.

Hernandez, M.J., Brennan, R.W. and Hawkins, R.A. Regional cerebral blood flow during neonatal asphyxia. In: Cerebral Metabolism and Neuronal Function, edited by J. V. Passonneau, R.A. Hawkins, W.D. Lust, and F.A. Welsh. Baltimore, MD: Williams & Wilkins, 1980, p.196-201.

Heymann, M.A., Payne, B.D., Hoffman, J.I.E., and Rudolph, A.M. Blood flow measurements with radionuclide labelled particles. Prog. Cardiovasc. Dis. 20:55-79, 1977.

Higganbottam, T: Cough induced by changes of ionic composition of airway surface liquid. Bull. Eur. Physiopathol. Respir. 20:553-562, 1984.

Hill, A. and Volpe, J.J. Measurement of intracranial pressure using the Ladd intracranial pressure monitor. J. Pediatr. 98:974-976, 1981

Himes, R.S., DiFazio, C.H. and Burney, R.G. Effects of lidocaine on the anesthetic requirements for nitrous oxide and halothane. Anesthesiology 47:437-440, 1977

Hinkle, A.J. Laryngoscopy in the awake neonate. Anesth. Rev. 13:43-46, 1986

Hooke, R. Phil. Trans. R. Soc. 1667, 2, 539

Hyman, A.L. The effects of lidocaine, hexamethonium and alpha and beta adrenergic blocking agents on the pulmonary veins in intact dogs. J. Pharmacol. Exp. Ther. 174:487-499, 1970

9 I

Inada, E., Cullen, D.J., Nemeskal, R.N. and Teplick, R. Effect of labetalol on the hemodynamic response to intubation: A controlled randomized double-blind study. Anesthesiology 67:A31, 1987

10 J

Jacobson, I., Harper, A.M. and McDowall, D.G. Relationship between venous pressure and cortical blood flow. Nature London 200:173-175, 1963

Jeffery, P.K., Korpas, J. and Widdicombe, J. G. Intraepithelial nerve fibres of the cat larynx and the expiration reflex.(Abstract) J. Physiol. (Lond.), 275: 35P- 36P, 1978.

Joels, N. and Neil, E. The excitation mechanism of the carotid body. Brit. Med. Bull. 19:21-24, 1963

Johnson, P.C. Review of previous studies and current theories of autoregulation. Circ. Res. 14-15, Suppl. 1:2-9, 1964

Johnson, P., Salisbury, D.M. and Storey, A.T. Apnea induced by stimulation of sensory receptors in the larynx. In Bosma, J.F. and Showacre, J.(eds): Development of upper respiratory anatomy and function. Washington, D.C., U.S. Government Printing Office, 1975,pp 160-178.

11 K

Katz, R.L. and Katz, G.J. Surgical infiltration of pressor drugs and their interactions with volatile anaesthetics. Br. J. Anaesth. 38:712-718, 1966

Kautto U-M. and Heinonen, J. Attenuation of circulatory response to laryngoscopy and tracheal intubation: a comparison of two methods of topical anaesthesia. Acta. Anaesthesiol. Scand. 26:599-602, 1982

Kautto, U-M. Attenuation of the circulatory response to laryngoscopy and intubation by fentanyl. Acta anaesth. scand. 26:217-221, 1982

Keats, A.S., D'Alessandro, G.L. and Beecher, H.K. A controlled study of pain relief by intravenous procaine. JAMA 147:1761-1763, 1951

Kelly, M.A. and Finer, N.N. Nasotracheal intubation in the neonate: Physiologic responses and effects of atropine and pancuronium. J. Pediatr. 105:303-309, 1984

Kety, S.S., Shenkin, H.A. and Schmidt, C.F. The effects of increased intracranial pressure on cerebral circulatory functions in man. Am. J. Physiol. 143:53-66, 1945

King, B.D., Harris, L.C., Greifenstein, F.E., Elder, J.E., and Dripps, R.D. Reflex circulatory responses to direct laryngoscopy and tracheal intubation performed during general anesthesia. Anesthesiology 12:556-566, 1951

Klein, S.W., Sutherland, R.I.L. and Morch, J.E. Haemodynamic effects of intravenous lidocaine in man. Can. Med. Assoc. J. 99:472-475, 1968

Kontos, H.A., Wei, E.P., Navari, R.M., Levasseur, J.E., Rosenblum, W.I. and Patterson Jr., J.L. Responses of cerebral arteries and arterioles to acute hypotension and hypertension. Am. J. Physiol. 234 (Heart Circ. Physiol. 3):H371-H383, 1978

Koppanyi, T. The sedative, central analgesic and anticonvulsant action of local anesthetics. Am J. Med. Sci. 244:646-654, 1962

Korpas,J., and Tomori,Z.: Cough and Other Respiratory Reflexes. Basel, S. Karger, 1979, pp 15 - 188.

Kurth, C.D., Wagerle, L.C. and Delivoria-Papadopoulos, M. Regional cerebral blood flow regulation by sympathetic nerves during seizures (Abstract). Federation Proc. 45:761, 1986

Kutas, H and Szabo, F. Effect of diarrhoea on the water and acid-base balance in piglets with enteric colibacillosis. Acta. Vet. Acad. Sci. Hung. 20:353-361, 1971

12 L

Lambert, G.F., Bogduk, N., Goadsby, P., Duckworth, J.W. and Lance, J.W. Decreased carotid arterial resistance in cats in response to trigeminal stimulation. J. Neurosurg. 61:307-315, 1984

Lambertsen, C.J., Kough, R.H., Cooper, D.Y., Emmel, G.L., Loeschke, H.H. and Schmidt, C.F. Oxygen toxity. Effects in man of oxygen inhalation at 1 and 3.5 atmospheres upon blood gas trnasport, cerebral circulation and cerebral metabolism. J. Appl. Physiol. 5:471-486, 1953

Lang, R. and Zimmer, R. Neurogenic control of cerebral blood flow. Exp. Neurol. 43:143-161, 1974

Laptook, A., Stonestreet, B., and Oh, W. The effects of different rates of plasmanate infusions upon brain blood flow after asphyxia and hypotension in newborn piglets. J. Pediatr. 100: 791-799, 1982.

Larsson, L.I., Edvinsson, L., Fahrenkrug, J., Hakanson, R., Owman, C., Schaffalitzky, O., Musckadell, D.E. and Sundler, F. Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves. Brain Res. 113:400-404, 1976

Lassen, N.A. Cerebral blood flow and oxygen consumption in man. Physiol. Rev. 39:183-238, 1959

Laurito, C.E., Baughman, V.L., Becker,G.L., Polek, V., Riegler, F.X. and Vadeboncouer, T.R. Effects of aerosolized and/or Intravenous Lidocaine on Hemodynamic responses to Laryngoscopy and Intubation in Outpatients. Anesth. Analg. 67:389-392, 1988

Leman,A.D., Straw,B., Glock, R.D., Mengeling,W.L., Penny, R.H.C., Scholl, E: Haematolgy and Chemistry values. Diseases of Swine 6th ed. Iowa State University Press, Ames, Iowa, USA. 1986, pp 27-43.

Lerman, J., Robinson, S., Willis, M.M. and Gregory, G.A. Anesthetic requirements for halothane in young children 0-1 month and 1-6 months of age. Anesthesiology 59:A446, 1983

Lescanic, M.L., Miller, E.D. and DiFazio, C.A. The effects of lidocaine on the whole body distribution of radioactively labeled microspheres in the conscious rat. Anesthesiology 55:269-272, 1981

Leslie, J.B., Kalayjian, R.W., McLoughlin, T.M., Russel, R.D., Plachetka, J.R. Ablation of the haemodynamic responses to tracheal intubation with preinduction intravenous Labetalol. Anesthesiology 67:A30, 1987

Lindgren, L. Comparison of halothane and enflurane anaesthesia for otolaryngological surgery in children. Br. J. Anaesth. 53:537-544, 1981

Lindgren, L. ECG changes during halothane and enflurane anaesthesia for ENT surgery in children. Br. J. Anaesth. 53:653-662, 1981

Lindgren, L. and Saarnivaara, L. Cardiovascular responses to enflurane induction followed by suxamethonium in children. Br. J. Anaesth. 55:269-274, 1983

Lindgren, L., Saarnivaara, L. and Himberg, J.-J. Comparison of oral triclofos, diazepam and flunitrazepam as premedicants in children undergoing otolaryngological surgery. Br. J. Anaesth. 52:283-290, 1980

Lindvall, M., Edvinsson, L.. and Owman, C. Sympathetic nervous control of cerebrospinal fluid production from the choroid plexus. Science 201:176-178, 1978

Long, U.S., Zebrowski, M.E. and Graney, W.F. Awake vs anesthetized intubation: A comparison of hemodynamic responses. Anesthesiology 57:A30, 1982

Lou, H.C, Lassen, N.A. and Friis-Hansen. Decreased cerebral blood flow after administration of sodium bicarbonate in the distressed newborn infant. Acta. Neurol. Scand. 57:230-247, 1978

Lou, H.C., Skov, H. and Pederson, H. Low cerebral blood flow: A risk factor in the neonate. J. Pediatr. 95:606-609, 1979

Lou, H.C., Lassen, N.A. and Friis-Hansen, B. Impaired autoregulation of cerebral blood flow in the distressed newborn. J. Pediatr. 96:118-121, 1979.

Lou, H.C., Lassen, N.A., Tweed, W.A., Johnson, G., Jones, G. and Palahniuk, R.K. Pressure passive cerebral blood flow and breakdown of the blood-brain barrier in experimental fetal asphyxia. Acta. Paeditr. Scand. 68:57-63, 1979.

Lundberg, J.M., Fahrenkrug, J. and Hokfelt, T. Co-exitence of peptide HI (PHI) and VIP in nerves regulating blood flow and bronchial smooth muscle tone in various mammals including man. Peptides 5:593-606, 1984

Lundberg, J., Brodin, E. and Saria, A. Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. Acta Physiol. Scand. 119:243-252, 1983

Lundberg, J., Hokfelt, T. and Nilsson, G. Peptide neurons in the vagus, splanchnic and sciatic nerves. Acta Physiol. Scand. 104:499-501, 1978

Lundberg, J and Saria, A. Capsaicin-sensitive vagal neurons involved in control of vascular permeability in rat trachea. Acta. Physiol. Scand. 115:521-523, 1982

13 M

MacEwan, W. Br. Med. J. 1880, 2, 122 (Reprinted in Survey of Anesthesiology 13:105, 1969)

MacKenzie, E.T., McCulloch, J., O'Keane, M., Pickard, J.D. and Harper, A.M. Cerebral circulation and norepinephrine: relevence of the blood-brain barrier. Am. J. Physiol. 231:483-488, 1976

Markowitz, S., Saito, K. and Moskowitz, M.A. Neurogenically-mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. J. Neurosurg. 7:4129-4136, 1987

Marcus, M.L., Bischoff, C.J. and Heistad, D.D. Comparison of microsphere and Xenon-133 clearance method in measuring skeletal muscle and cerebral blood flow. Circ. Res. 48:748-761, 1981

Marcus, M.L. and Heistad, D.D. Effects of sympathetic nerves on cerebral blood flow in awake dogs. Am. J. Physiol. 236 (Heart. Circ. Physiol.5:) H549-H553, 1979

Matthew, N.J., Meyer, J.S., Bell, R.L., Johnson, P.C. and Neblett, C.R. Regional cerebral blood flow and blood volume measured with the gamma camera. Neuroradiology 4:133-140, 1972

Mayhan, W.G., Faraci, F.M. and Heistad, D.D. Disruption of the blood brain barrier in cerebrum and brain stem during acute hypertension. Am. J. Physiol. 20:H1171-1175, 1986

McDonald, M.M., Koops, B.L., Johnson, M.L., Guggenheim, M.A., Rumack, C.M., Mitchell, S.A. and Hathaway, W.E. Timing and antecedents of intracranial hemorrhage in the newborn. Pediatr. 74:32-36, 1984.

McHenry, L.C. Jr., Slocum, H.C., Bivens, H.E., Mayes, H.A. and Heyes, G.I. Hyperventilation in awake and anesthetised man. Arch. Neurol. 12:270-277, 1965

McLesky, C.H., Cullen, B.F., Kennedy, R.D. and Galindo, A. Control of cerebral perfusion pressure during induction of anaesthesia in high risk neurosurgical patients. Anesth. Analg. 53:985-992, 1974

Ment, L.R., Stewart, W.B., and Duncan, C.C. Beagle puppy model of intraventricular hemorrhage. J. Neurosurg. 57: 219-223, 1982.

Meyer, J.S., Ishihara, N., Deshmukh, V.D., Haritomi, H.,Sakai, F., Hsu, M-C. and Pollack, P. Improved method for non-invasive measurement of regional cerebral blood flow by xenon-133 inhalation. Description of method and normal values obtained in healthy volunteers. Stroke 9:195-205, 1978

Mirakhur, R.K. Bradycardia with laryngeal spraying in children. Acta. Anaesth. Scand. 26:130-132, 1982

Misfeldt, B.B., Jorgensen, P.B. and Risoj, M. The effect of nitrous oxide and halothane upon the intracranial pressure in hypocapnic patients with intracranial disorders. Br. J. Anaesth. 46:853-858, 1974

Morita, H., Nemota, E.M., Bleyaert, A.L. and Stezoski, S.W. Brain blood flow autoregulation and metabolism during halothane anesthesia in monkeys. Am. J. Physiol. 233:H670-H676, 1977

Moskowitz, M.A., Wei, E.P., Saito, K. and Kontos, H.A. Trigeminalectomy modifies pial arteriolar responses to hypertension or norepinephrine. Am. J. Physiol. 255 (Heart Circ. Physiol. 24);H1-H6, 1988

Moss, E., Powell, D., Gibson, R.M. and McDowall, D.G. The effects of tracheal intubation on intracranial pressure following induction of anaesthesia with thiopentone or althesin in patients undergoing neurosurgery. Br. J. Anaesth. 50:353-360, 1978

14 N

Nadel, J.A. and Widdicombe, J.G. Effect of changes in blood gas tensions and carotid sinus pressure on tracheal volume and total lung resistance to airflow. J. Physiol. (Lond.) 163:13-33, 1962

Newhouse, M.T., Becklake, M.R., Macklem, P.T. and McGregor, M. Effect of alteration in end-tidal CO2 tension on flow resistance. J. Appl. Physiol. 19:745-749, 1964

Nicodemus, H.F., Rahini, C.N., Bachman, L. and Smith T.C. Median effective doses ED50 of halothane in adults and children. Anesthesiology 31:344-348, 1969

Nielsen, K.C. and Owman, C.H. Adrenergic innervation of pial arteries related to the Circle of Willis in the cat. Brain Res. 6:773-776, 1967

15 O

Oh, W. Personal communication.

Owman, C., Edvinsson, L. and Nielsen, K.C. Autonomic neuroreceptors in brain vessels. Blood Vessels II: 2-31, 1974

16 P

Pelton, D.A., Daly, M., Cooper, P.D. and Conn, A.W. Plasma lidocaine concentrations following topical aerosol application to the trachea and bronchi. Canad. Anaesth. Soc. J. 17:250-255, 1970

Perez-Hernandez, M.J. and Anderson, D.K. Autoregulation of cerebral blood flow and its relation to cerebrospinal fluid pH. Am. J. Physiol. 231:929-935, 1976

Perlman, J.M., Goodman, S., Kreusser, K.L. Volpe, J.J. Reduction in intraventricular hemorrhage by elimination of fluctuating cerebral blood-flow velocity in preterm infants with respiratory distress syndrome. N. Engl. J. Med. 312:1353-1357, 1985

Perlman, J.M. and Volpe, J.J. Suctioning in the preterm infant: effects on cerebral blood flow velocity, intracranial pressure, and arterial blood pressure. Pediatrics 72:329-334, 1983

Pickhard, J.D. and MacKenzie, E.T. Inhibition of prostaglandin synthesis and the response of baboon cerebral circulation to carbon dioxide. Nature London New Biol. 245: 187-188, 1973

Pierce, E.C., Lambertsen, C.J., Deutsch, S., Chase, P.E., Linde, H.W., Dripps, R.D and Price, H.L. J. Clin. Invest. 41:1664-1671, 1962

Plum F, Posner, J.B. and Troy, B. Cerebral metabolic and circulatory responses to induced convulsions in animals. Arch. Neurol. 18:1-13, 1968

Pon, W.G. and Haupt, K.A. The biology of the pig. Ithica. Cornell University Press, 1978, p. 99

Poulton, T.J. and James, F.M. III. Cough suppression by lidocaine. Anesthesiology 50:470-472, 1979

Prys-Roberts, C., Greene, L.T., Meloche, R. and Foex, P. Studies of anaesthesia in relation to hypertension. 11 Haemodynamic consequences of induction and endotracheal intubation. Br. J. Anaesth. 43:531-547, 1971

Purves, M.J. The Physiology of the Cerebral Circulation. New York: Cambridge Univ. Press, 1972, p. 1-414.

Purves, M.J. Do vasomotor nerves significantly regulate cerebral blood flow? Circ. Res. 43:485-493, 1978

Purves, M.J. and Biscoe, T.J. Development of chemoreceptor activity Brit. Med. Bull. 22:56-60, 1966

17 R

Raisis, J.F., Kindt, G.W., McGillicuddy, J.E. and Gianotta, S.L. The effects of primary elevation of cerebral venous pressure on cerebral haemodynamics and intracranial pressure. J. Surg. Res. 26: 101-107, 1979

Raju, T.N.K., Doshi, U.V. and Vidyasagar, D. Cerebral perfusion pressure studies in healthy preterm and term newborn infants. J. Pediatr. 100:139-142, 1982

Raju, T.N.K., Vidyasagar, D., Torres, C., Grundy, D and Bennet, E.J. Intracranial pressure during anesthesia and intubation in infants. J. Pediatr. 96:860-862, 1980

Rapela, C.E. and Green, H.D. Autoregulation of canine cerebral blood flow. Circ. Res. 15 (Suppl.):205-212, 1964

Reid, L.C. and Brace,D.E. Irritation of the respiratory tract and its reflex effect on the heart. Surg. Gynecol and Obstet. 70: 157-162, 1940

Reivich, M. Arterial PCO2 and cerebral haemodynamics. Am. J. Physiol. 206:25-35, 1964

Rosenberg, A.A., Jones, D.M. Jnr., Traytsman, R.J., Simmons, M.A. and Molteni, R.A. Respone of cerebral blood flow to changes in pCO2 in fetal, newborn and adult sheep. Am. J. Physiol. 242:H862-H866, 1982

Rosenberg, P.H., Heinonen, J. and Takasaki, M. Lidocaine concentration in blood after topical anaesthesia of the upper respiratory tract. Acta. Anaesth. Scand. 24:125-128, 1980

Rowbotham, E.S. Intratracheal anaesthesia by the nasal route for operations on the mouth and lips. Br. Med. J. 2:590-591, 1920

Russel, W.J., Morris, R.G., Frewin, D.B. and Drew, S.E. Changes in plasma catecholamine concentrations during endotracheal intubation. Br. J. Anaesth. 53:837-839, 1981

18 S

Said, S.I. and Mutt, V. Isolation from porcine intestine of a vasoactive octocosapeptide related to secretin and glucagon. Eur. J. Biochem. 28:199-204, 1972

Sakabe, T., Maekawa, T., Ishikawa, T. and Takeshita, H. The effects of lidocaine on canine cerebral metabolism and circulation related to the electroencephalogram. Anesthesiology 40:433-441, 1974

Samra, S.K., Deutsch, G. and Arens, J.F. Effect of nitrous oxide on global and regional cerebral blood flow in humans. Anesthesiology 69:A536, 1988

Schmidt, C.F. The cerebral circulation in health and Disease. Springfield, IL: Thomas, 1950, pp 1-86.

Sell, T.L., Croughwell, N., Camporesi, E., Jones, R.H. and Reves, J.G. Assessment of wall motion by radionuclide angiocardiography during endotracheal intubation. Anesthesiology 67:A27, 1987

Shapiro, H.M., Greenberg, J.H., Van Horn Naughton, K. and Reivich, M. Heterogeniety of local cerebral blood-flow-PaCO2 sensitivity in neonatal dogs. J. Appl. Physiol. 49:113-118, 1980

Shapiro, H.M., Whyte, S.R., Harris, A.B. and Galindo, A. Acute intraoperative intracranial hypertension in neurosurgical patients. Anesthesiology 37:399-405, 1972

Shimbourne, E.A., Vapaavuori, E.K., Williams, R.L., Heymann, M.A. and Rudolph, A.M. Development of baroreflex activity in unanaesthetised fetal and neonatal lambs. Circ. Res. 31:710-718, 1972

Shribman, A.J., Smith, G. and Achola, K.J. Cardiovascular and catecholamine responses to laryngoscopy with and without tracheal intubation. Br. J. Anaesth. 59:295-9, 1987

Siedlecki, J. Disturbances in function of the cardiovascular system in patients following endotracheal intubation and attempts of their prevention by pharmacological blockade of sympathetic system. Anaesth. Resus. Intens. Therap. 3:107-123, 1975

Siegal, S. Nonparametric statistics for the behavioural sciences. McGraw Hill, New York, 1956

Simbruner, G., Coradello, H., Fodor, M., Havelec, L., Lubec, G. and Pollak, A. Effect of tracheal suction on oxygenation, circulation, and lung mechanics in newborn infants. Arch. Dis. Child. 56:326-330, 1981

Snedecor, G.W. and Cochran, W.G. Statistical Methods. Iowa State University Press, Ames, 1980

Stanley, T.H. and Webster, L.R. Anesthetic requirements and cardiovascular effects of fentanyl-oxygen and fentanyl-diazepam-oxygen anesthesia in man. Anesth. Analg. 57:411-416, 1978

Steinhaus, J.E. and Gaskin, L. A study of intravenous lidocaine as a suppressant of cough reflex. Anesthesiology 24:285-290, 1963

Stephen, C.R., Woodhall, B., Golden, J.B., Martin, R. and Nowill, W.K. The influence of anesthetic drugs and techniques on intracranial tension. Anesthesiology 15:365-377, 1954

Stewart, W.B. Blood flow and metabolism in the developing brain. Semin. Perinat. 11:112-116, 1987

Stoelting, R.K. Circulatory changes during direct laryngoscopy and tracheal intubation: Influence of duration of laryngoscopy with or without prior lidocaine. Anesthesiology 47:381-384, 1977

Stoelting, R.K. Blood pressure and heart rate changes during short-duration laryngoscopy for tracheal intubation: Influence of viscous or intravenous lidocaine. Anesth. Analg. 57:197-199, 1978

Stoelting, R.K. Attenuation of blood pressure response to laryngoscopy and tracheal intubation with sodium nitroprusside. Anesth. Analg 58:116-119, 1979

Stoelting, R.K. and Peterson, C. Circulatory changes during anesthetic induction: impact of d-tubocurarine pretreatment, thiamylal, succinylcholine, laryngoscopy, and tracheal lidocaine. Anesth. Analg. 55:77-81, 1976

Stoelting, R.K., Gibbs, P.S., Creasser, C.W. and Peterson, C. Hemodynamic and ventilatory responses to fentanyl, fentanyl-droperidol and nitrous oxide in patients with acquired valvular heart disease. Anesthesiology, 42:319-324, 1975

Stonestreet, B.S., Goldstein, M., Piva, D. and Barefield, E. Autoregulation of brain blood flow during hypercarbia in piglets. Ped. Res. 12:468A, 1985

Storey,A.T. Interactions of alimentary and upper respiratory tract reflexes. In Sessle,B.J., and Hannam, A.G.(eds.): Mastication and Swallowing. Toronto and Buffalo, University of Toronto Press, 1976, pp 22-31.

Stow, P.J., McLeod, M.E., Burrows, F.A. and Creighton, R.E. Anterior fontanelle pressure responses to tracheal intubation in the awake and anaesthetised infant. Br. J. Anaesth. 60:167-170, 1988

Strong, M.S., Waughan, C.W. and Mahler, D.L. Cardiac complications of microsurgery of the larynx: Etiology, incidence, and prevention. Laryngoscope, 84:908-920, 1974

Sullivan, C.E., Murphy, E, Kozarl,F. Waking and ventilatory responses to laryngeal stimulation in sleeping dogs. J. Appl. Physiol. 45:681-689, 1978

Sumi, T. Coordination of neural organization of respiration and deglutition. Its change with postnatal maturation. In Bosma. J.F. and Showacre, J. (Eds.): Development of upper respiratory anatomy and function. Washington. D.C., U.S. Government Printing Office, 1975, pp. 145-157

Suzuki, M. and Kirchner, J.A. Laryngeal reflex pathways related to rate and rhythm of the heart. Ann. Otol. Rhinol. Laryngol. 76:774-790, 967

19 T

Takeshima, K., Noda, K. and Higaki, M. Cardiovascular response to rapid anesthesia induction and endotracheal intubation. Anesth. Analg. 43:201-208, 1964

Takashima, J. and Tanaka, K. Microangiopathy and vascular permeability of the subependymal matrix in the premature infant. Can. J. Neurol. Sci. 5:45-50, 1978

Takeshita, H. and Okuda, Y. The effects of ketamine on cerebral ciculation and metabolism in man Anesthesiology 36:69-75, 1972

Tam, S., Chung, F. and Campbell, M. Intravenous lidocaine: Optimal time of injection before tracheal intubation. Anesth. Analg. 66:1036-1038, 1987

Tatemoto, K. and Mutt, V. Isolation and characterization of the intestinal peptide porcine PHI (PHI-27), a new member of the glucagon-secretin family. Proc. Natl. Acad. Sci. USA 78:6603-6607, 1981

Toda, N. and Fujita, Y. Responsiveness of isolated cerebral and peripheral arteries to serotonin, norepinephrine and transmural electrical stimulation. Circ. Res. 33:98-104, 1973

Tomori, Z. and Widdicombe, J.G. Muscular, bronchomotor, and cardiovascular reflexes elicited from mechanical stimulation of the respiratory tract. J. Physiol. (London), 200:25-50, 1969

Towart, R. The selective inhibition of serotonin induced contractions of rabbit cerebral vascular smooth muscle by calcium-antagonistic dihydropyridines. An investigation of the mechanisms of action of nimodipine. Circ. Res. 48:650-657, 1981

20 U

Uddman, R., Sundler, F. and Emson, P. Occurrence and distribution of neuropeptide-Y-immunoreactive nerves in the respiratory tract and middle ear. Cell Tissue Res. 237:321-327, 1984

21 V

Vasquez, J. and Purves, M.J. The cholinergic pathway to cerebral blood vessels. I. Morphological studies. Pfluegers Arch. 379:157-163, 1979

Venus, B., Polassani, V. and Pham, C.G. Effects of aerosolized lidocaine on circulatory responses to laryngoscopy and tracheal intubation. Crit. Care. Med. 12:391-394, 1984

Viegas, O. and Stoelting, R. K. Lidocaine in arterial blood after laryngotracheal administration. Anesthesiology 43:491-493, 1975

Von Euler, U.S. Liljestrand, G. and Zotterman, Y. The excitation mechanism of the chemoreceptors of the carotid body. Skand. Arch. Physiol. 83:132-138, 1939

22 W

Wagerle, L.C. and Delivoria-Papadopoulos, M. Alpha adrenergic receptor subtypes in the cerebral circulation of newborn piglets. Am. J. Physiol. 252 (Regulatory Integrative Comp. Physiol. 21):R1092-R1098, 1987

Wagerle, L.C., Kumar, S.P. and Delivoria-Papadopoulos, M. Effect of sympathetic nerve stimulation on cerebral blood flow in newborn piglets. Pediatr. Res. 20:131-135, 1986

Wagerle, L.C., Kumar, S.P. and Delivoria-Papadopoulos, M. Sympathetic nerve stimulation attenuates cerebral blood flow (CBF) in hypercapnic newborn piglets. Pediatr. Res. (Abstract) 81:384A, 1984

Wagerle, L.C., Mishra, O.P. and Delivoria-Papadopoulos, M. Role of cyclo-oxygenase pathway in the cerebral arteriolar response to hypoxia in newborn piglets. (Abstract) Pediatr. Res. 83:360A, 1985

Wagerle, L.C. and Mishra, O.P. Mechanism of CO2 response in cerebral arteries of the newborn pig: Role of phospholipase, cyclo-oxygenase and lipoxygenase pathways. Circ. Res. 62:1019-1026, 1988

Wagner, E.M. and Traystman, R.J. Cerebral venous outflow and arterial microsphere flow with elevated venous pressure. Am. J. Physiol. 244:H505-H512, 1983

Wahl,M. and Kuchinsky, W. Unimportance of perivascular H+ and K+ activities for the adjustment of pial arterial diameter during changes of arterial blood pressure in cats. Pfleugers Arch. 382:203-208, 1979

Wedley, J. Br. J. Clin. Equip. Letter 4:49, 1979

Wei, E.P., Ellis, E.F. and Kontos, H.A. Role of prostaglandins in pial arteriolar responses to CO2 and hypoxia. Am. J. Physiol. 238(Heart Circ. Physiol. 7):H226-H230, 1980

Widdicombe, J.G. Respiratory reflexes and defense. In Brain, J.D., Proctor, D.E.,and Reid, L,M. (Eds.): Respiratory Defense Mechanisms. Part 11. New York and Basel, Marcel and Decker, 1977, pp 593-630.

Widdicombe, J. G.: Nervous receptors in the respiratory tract and lungs. In Hornbein, T.F. (ed): Regulation of Breathing. Part 1. New York and Basel. Marcel Decker, 1981, pp 429-472

Widdicombe, J.G. and Sterling, G.M. The autonomic nervous system and breathing. Arch. Intern. Med. 126:311-323, 1970

Wiggers,C.J. On the action of adrenaline on cerebral vessels Am. J. Physiol. 14: 452-465, 1905.

Wiklund, L. Human hepatic blood flow and its relation systemic circulation during intravenous infusion of lignocaine. Acta. Anesthesiol. Scand. 21:148-160, 1977

Winer, B.J. Statistical principles in experimental design. McGraw Hill, Kogakusha, 1971

Winn, H.R., Rubio, R. and Berne, R.M. Brain adenosine concentration during hypoxia in rats. Am J. Physiol. 241 (Heart Circ. Physiol. 10):H235-H242, 1981

Winn, H.R., Welsh, J.E., Rubio, R. and Berne, R.M. Brain adenosine concentration production in rat during sustained alteration in systemic blood pressure. Am J. Physiol. 239 (Heart Circ. Physiol. 8):H636-H641, 1980

Wolff, H.G. and Lennox, W.G. Cerebral circulation. The effect on pial vessels of variation of the oxygen and carbon dioxide content in the blood. Arch. Neurol. Neurosurg. Psychiatry 32:1097-1120, 1930

Wollman, H., Smith, A.L., Alexander, S.C. Effects of general anesthetics in man on the ratio of cerebral blood flow to cerebral oxygen consumption. Cerebral blood flow clinical and experimental results. Brock, M., Fieschi, C., Ingvar, D.H. (Eds.), New York, Springer-Verlag, 1969, p.241-243

Wollman, H., Alexander, S.C., Cohen, P.J., Chase, P.E., Melman, E. and Behar, M.G. Cerebral circulation of man during halothane anesthesia. Anesthesiology 25:180-184, 1964

Wycoff, C.C. Endotracheal intubation: effects on blood pressure and pulse rate. Anesthesiology 21:153-158, 1960

23 Y

Yakaitis, R.W., Blitt, C.D. and Angiulo, J.P. End-tidal halothane concentration for endotracheal intubation. Anesthesiology, 47:386-388, 1977

Figure P2-1: Screen display showing the response to laryngoscopy/ spray, and laryngoscopy/intubation in an animal sprayed with saline. Note the more pronounced and more prolonged pressure response following laryngoscopy/intubation.

Figure P2-2: Heart rate response following laryngoscopy/ intubation. The arrow marks the beginning of the stimulus. The deep dips marked by symbols indicate microsphere injections.

Figure P2-3: Note the bradycardic response seen in this animal both during the laryngoscopy/spray, and following intubation.

Screen display

Figure P3-1: Peripheral oxygen saturation response to laryngoscopy/ intubation. The arrow marks the beginning of the laryngoscopy, and the diamond marks the intubation timepoint.